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Mucoadhesive polymers in the design of nano-drug delivery systems for administration by non-parenteral routes: A review

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ABSTRACT

The presence of a mucus layer that covers the surface of a variety of organs has been capitalized to develop mucoadhesive dosage forms that remain in the administration site for prolonged times, increasing the local and/or systemic bioavailability of the administered drug. The emergence of micro and nanotechnologies together with the implementation of non-invasive and painless administration routes has revolutionized the pharmaceutical market and the treatment of disease. Aiming to overcome the main drawbacks of the oral route and to maintain patient compliance high, the engineering of innovative drug delivery systems administrable by mucosal routes has come to light and gained the interest of the scientific community due to the possibility to dramatically change pharmacokinetics. In addition, to achieve the goal of mucosal drug administration, the development of biomaterials has been refined to fit specific applications. The present review initially describes the potential of nano-drug delivery systems conceived for mucosal administration by

Abbreviations: ABC, ATP-binding cassette super family pump; AIDS, acquired immunodeficiency syndrome; ALG, alginate; APBA, 3-aminophenyl boronic acid; API, active pharmaceutical ingredient; BBB, blood–brain barrier; BCSFB, blood–cerebrospinal fluid barrier; BPI-3, para-influenza type 3 virus; BRB, blood–retinal barrier; CMC, carboxymethyl cellulose; CaCMC, carboxymethyl cellulose calcium salt; NaCMC, carboxymethyl cellulose sodium salt; CNS, central nervous system; CS, chitosan; CSF, cerebrospinal fluid; DDS, drug delivery system; DOPA, dihydroxyphenyl-L-alanine; EDTA, ethylenediaminetetraacetic acid; G, α-L-guluronic acid; GalM, galactomannan; GIT, gastrointestinal tract; GluM, glucomannan; GRAS, generally recognized as safe; HSV-2, Herpes simplex virus type 2; HIV, human immunodeficiency virus; HEC, hydroxyethyl cellulose; HPC, hydroxypropyl cellulose; HPMC, hydroxypropyl methylcellulose; IBD, inflammatory bowel disease; i.n., intranasal administration route; i.v., intravenous administration route; LLRs, lectin-like receptors; LMWH, low molecular weight heparin; M, β-D-mannuronic acid; MC, methylcellulose; MLV, multilamellar vesicle; MPI, material of pharmaceutical interest; MPT, multiple-particle tracking; MST, materials science tetrahedron; Nano-DDS, nano-drug delivery system; NMR, nuclear magnetic resonance spectroscopy; NPs, nanoparticles; PAA, poly(acrylic acid); PAMAM, poly(amidoamine); PBCA, poly(n-butyl cyanoacrylate); PCL, poly(epsilon-caprolactone); PEG, poly(ethylene glycol); PEI, poly(ethylene imine); PEO, poly(ethylene oxide); PEO-PPO, poly(ethylene oxide)-co-poly(propylene oxide) block copolymer; PHEMA, poly(hydroxyethyl methacrylate); PK, pharmacokinetics; PLA, poly(lactic acid); PLGA, poly(lactic-co-glycolic acid); PMAA, poly(methacrylic acid); PMMA, poly(methyl methacrylate); PMS, pharmaceutical materials science; PR&D, pharmaceutical research and development; PVP, poly(vinyl pyrrolidone); RGD, arginine-glycine-aspartic acid; SLNs, solid lipid nanoparticles; TB, tuberculosis; TMC, trimethyl chitosan; US FDA, US Food and Drug Administration.

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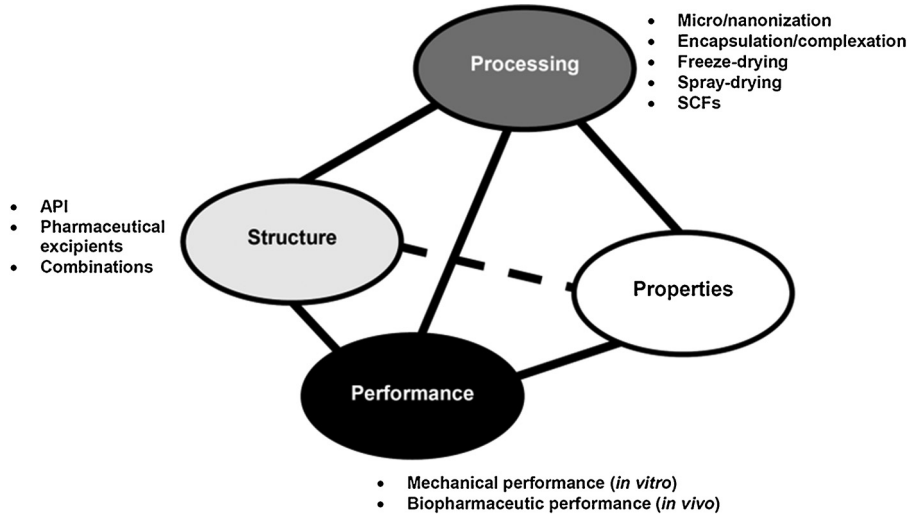
diverse non-parenteral routes (*e.g.*, oral, inhalatory, *etc.*). Then, the benefit of the incorporation of mucoadhesive polymers into the structure of these innovative pharmaceutical products to prolong their residence time in the administration site and the release of the drug cargo will be discussed with focus in the developments of the last decade. In addition, the regulatory status of the most extensively used mucoadhesive polymers will be emphasized. Finally, a thorough overview of the different pharmaceutical applications of mucoadhesive polymers will be addressed.

Contents

1. Introduction and scope

The main goal of pharmaceutical research and development (PR&D) is to design products with ensured quality to effectively treat disease [1]. Patient and clinician compliance are crucial to the successful bench-to-bedside translation [1]. Aiming to make this process more rational, coherent, efficient and cost-effective, the field of Pharmaceutical Materials Science (PMS) has emerged as the "*study of the physical properties and behavior of materials of pharmaceutical interest in relation to the product performance*" [2]. Materials of pharmaceutical interest (MPIs) are classified into two main classes, namely active pharmaceutical ingredients (API) and non-pharmacologically active excipients [2]. The former are entailed to trigger a pharmacological response, while the latter are incorporated into the formulation to improve its (bio)pharmaceutical properties and performance. In this context, PMS embraced the materials science tetrahedron (MST) (Scheme 1) and pursues the thorough characterization and understanding of the structure–properties relationship of all the components in a pharmaceutical product (including the pure drug) and the development of appropriate processing methods (*e.g.*, micronization, nanonization, freeze-drying and spray-drying) that ensure a predictable performance *in vitro* (*e.g.*, tablet mechanical properties, disintegration and drug dissolution) and *in vivo* (*e.g.*, bioavailability) [3,4].

One of the challenges in early and late PR&D pertains to the poor aqueous solubility and permeability of drugs [5]. This property is common to approximately 50% of the APIs on the market and it represents a crucial hurdle during the



Scheme 1. Pharmaceutical materials science (PMS) tetrahedron.

stages of drug product development [5,6]. Moreover, low solubility in biological fluids leads to limited absorption in the gastrointestinal tract (GIT) and limited bioavailability, the oral route being the most popular one [7]. Solubility is an intrinsic property that depends on the nature of the molecule, whereas dissolution is an extrinsic one that can be modified by different means such as reduction of drug particle size [4,8] and encapsulation in a variety of micro and nanocarriers [9].

PMS was initially implemented to improve the performance of already approved drugs [4]. However, drug candidates in the pipeline are becoming more complex structures usually highly hydrophobic and this nature jeopardizes not only the conduction of more advanced preclinical and clinical trials but also preliminary high-throughput screening assays *in vitro* [10]. For example, to assess the antiviral activity of a new compound in cell culture, it needs to be soluble in the culture medium. Thus, the early characterization of the solubility and other physicochemical parameters has become a crucial step in the whole PR&D process [11–16] to increase the translatability of new chemical entities and to reduce the drug attrition rates.

As stated before, most of the pharmaceutical products are solids intended for oral administration [7]. This route is usually associated with hepatic first pass metabolism, chemical and enzymatic degradation in the GIT medium, basolateral-to-apical efflux by pumps of the ATP-binding cassette superfamily (ABCs) and reduced bioavailability. The most straightforward strategy to circumvent these disadvantages is the parenteral route. However, it induces tissue damage, pain and patient incompliance [17]. Moreover, systemic exposure often leads to adverse effects that cannot be easily controlled. The oral route is also less feasible when more prolonged release kinetics is demanded owing to the short gastric emptying and intestinal transit times [18].

Among the different approaches pursued to optimize the physicochemical and (bio)pharmaceutical performance of drugs, the presence of a mucus layer that covers the surface of a variety of organs has been capitalized to develop mucoadhesive dosage forms that remain in the administration site for more prolonged times, increasing the local and/or systemic bioavailability of the administered drug [19]. The emergence of micro and nanotechnologies together with the implementation of non-invasive and painless administration routes has revolutionized the pharmaceutical market and the treatment of disease [7,20–24]. Aiming to overcome the main drawbacks of the oral route and to maintain patient compliance high, the engineering of innovative drug delivery systems (DDS) administrable by mucosal routes has come to light and gained the interest of the scientific community due to the possibility to dramatically change pharmacokinetics [25]. In addition, to achieve the goal of mucosal drug administration, the development of biomaterials has been refined to fit specific applications.

The present review initially describes the potential of nano-DDS conceived for mucosal administration by diverse non-parenteral routes (*e.g.*, oral, inhalatory, *etc.*). Then, the benefit of the incorporation of mucoadhesive polymers into the structure of these innovative pharmaceutical products to prolong their residence time in the administration site and the release of the drug cargo will be discussed with focus in the developments of the last decade. In addition, the regulatory status of the most extensively used mucoadhesive polymers will be emphasized. Finally, a thorough overview of the different pharmaceutical applications of mucoadhesive polymers will be addressed.

2. Molecular features of mucosae

Mucosae, and in particular mucosal fluids, are an essential part of mucoadhesive phenomena and thus deserve special attention when addressing the subject. Mucosal tissues cover natural body cavities, providing an epithelial barrier to the external environment. From a histological point of view, the mucosa is composed by (from the lumen

Table 1
Main features of different mucosal sites.

Mucosae	Epithelium type[a]	Temperature	Typical shear stress	Mucus			
				Mucins concentration	pH[b]	Clearance/production time or rate	Mean layer thickness
Buccal	Stratified squamous non-keratinized[c]	36.5 °C [50]	L (resting)/M (mastigation)	0.1–0.5% [35,51]	7.1 (6.8–7.4)	0.1–1.85 mL/min [52]	10–100 μm [52,53]
Esophageal	Stratified squamous non-keratinized	37 °C	L (resting)/M (deglutition)	0.1–0.3%[d] [54]	6 (4–7)[e] [55]	0.2–0.3 mg/cm^2/min[f] [56]	95 μm [56,57]
Gastric	Simple columnar	37 °C	L (resting)/M (digestion)	3% [58]	1.6 (1–0-2.5)[g] to 7.2 (7.1–7.3)[h]	4–5 h [59]	180 μm (50–450 μm) [60]
Small intestinal	Simple columnar (with villi)	37 °C	L (resting)/M (digestion)	1% [61]	6.9 (5.9–7.5)	47–270 min[i] [62]	0–37 μm [32,35]
Colonic	Simple columnar	37 °C	L	<5% [35]	6.6 (6.0–7.0)	≈270–300 min[i] [63]	110–130 μm (50–275 μm) [64]
Rectal	Simple columnar	37 °C [50]	L (resting)/M (defecation)	<5% [35]	7.5 (6.8–7.9) [65]	3–4 h[j] [66]	150 μm (75–250 μm) [64]
Nasal	Pseudostratified ciliated columnar	30 °C (28–34 °C) [67,68]	M (respiration)/H (coughing)	2–3% [69,70]	6.6 (6.3–6.7)	5–10 min [71,72]; 0.5–1 mL/min [73]	10–15 μm [74]
Lung[k]	Variable[l]	32–36 °C[m] [75]	M (respiration)/H (coughing)	2–4% [76]	7.0[n]	5–10 cm/min [35]	5–55 μm [32]
Ocular[o]	Stratified squamous non-keratinized	34 °C[p] [77,78]	H (blinking)	≈0.01%[q] [79]	7.6 (7.5–7.8)[r]	5–10 s[r] [80]	3–5 μm[r,s] [81–83]
Vaginal[t]	Stratified squamous non-keratinized	37 °C [84]	L (resting)/H (copulation)	1–2%[u] [29]; 5%[v] [85]	4.2 (3.5–4.5)[w] [86]	6 mL/d[x] [84]; 1.5 mL/d[y] [85]	20 μm[z] [87]

[a] From reference [28].
[b] Mean values (mean range), as recently reviewed in reference [43], except where noted otherwise.
[c] Except at hard palate and gingival where the epithelium is partially keratinized.
[d] Estimation from mucin recovered by esophageal perfusions with normal saline.
[e] Lower than 4 in gastroesophageal reflux.
[f] Expressed as mucin production.
[g] In the apical layer.
[h] In the basal layer.
[i] Rat model.
[j] *Ex vivo* data from human rectal biopsies.
[k] Conduction lung (includes secondary bronchi, bronchioles and terminal bronchiole).
[l] Pseudostratified ciliated columnar (secondary bronchi), simple columnar to simple cuboidal (bronchioles) or simple cuboidal (terminal bronchiole).
[m] In bronchi and dependent on breathing rate and environmental temperature.
[n] In bronchi.
[o] Considering the fibrous tunic, which comprises the cornea and the sclera.
[p] At the cornea.
[q] Estimated content in tear film.
[r] Values for tear film.
[s] 10% of the tear film thickness has been attributed to the actual mucus layer [33].
[t] Typical values for healthy women during reproductive years.
[u] In vaginal fluid (diluted upon sexual stimulation [88]).
[v] In cervical mucus.
[w] In vaginal fluid and can be significantly increased during vulvovaginal infection (5–6.5), in menopause (6–7.5) or when semen is present (7–8.5) as reviewed in [29] and [84].
[x] Value for vaginal fluid (considerably increased with sexual stimulation [88]).
[y] Value for cervical mucus.
[z] Calculated mean value based on the volume of fluid present in the vagina and its total surface area (previously estimated at several tens of micrometers [32] and considerably increased with sexual stimulation [88]).
N.A., data not available; L, low; M, moderate; H, high; d, day.

to the submucosa) an epithelial layer, which can be of different types (Table 1), the lamina propria and, at the GIT, the muscularis mucosae. Alongside variations in epithelial type, the presence and/or distribution of carbohydrate moieties at the glycocalyx, and roughness or folding features of mucosal surfaces may also differ, thus influencing mucoadhesion phenomena [26]. Another important issue has to deal with shearing at mucosal sites (Table 1). Moderate values can promote interaction of DDS with mucosae/mucus fluids and favor adhesion but, in cases where shear stress is too high, the time for the consolidation of adhesive bonds may not be long enough.

In these cases, rapid washing-out and shear-thinning of mucoadhesive systems is a considerable obstacle to be surpassed when developing drug carriers to be administered in anatomical sites such as the ocular surface.

One defining feature of mucosae is the presence of a protective layer of fluid called mucus, which acts as a physical barrier to chemical and biological insult, as well as a natural lubricant opposing shear damaging. This fluid also plays important homeostatic functions, namely in regulating water balance and ion transport, clearing of cellular debris, mucosal immune-regulation, transporting sperm in the cervicovaginal tract, among others [27]. Mucus is produced by specialized goblet cells or glands at the mucosa/sub-mucosa, except in the case of the stomach (mucus is produced by epithelial cells) and the vagina [28]. In the last case, vaginal fluid results from the mixture of different liquids including mucus produced at the cervix [29]. Mucus is a highly hydrated (≥95% water) non-Newtonian, viscoelastic system comprising a tridimensional network of randomly entangled mucins (2–5% of its mass), and presenting typical viscosity values in the range of 10–10^3 Pa.s at low shear rate but quite variable depending on particular composition, anatomical site and physiophathological conditions (Table 1) [30]. The width of the mesh spaces delimited by mucin fibers has been previously estimated to be around 20–200 nm [31], though recent studies indicate that particles as large as 500 nm in diameter can still diffuse through mucus as long as adhesive interactions are minimal (reviewed in [32]). Indeed, a recent study showed that the random distribution and entanglement of mucins leads to substantial heterogeneity in the mucus mesh diameter (50–1800 nm) [33].

Mucins present in mucus are directly involved in adhesion phenomena. The term "mucins" is usually applied in order to include a somewhat heterogeneous group of glycoproteins that are coded by the *MUC* gene family but differ in their glycosylation and polypeptide sequences [34]. Secretory (or soluble) mucins are heterogeneous high molecular weight (10^6–10^7 g/mol and several micrometers in length) glycoproteins (≈75% carbohydrate/25% amino acid residues linked *via O*-glycosidic bonds between *N*-acetylgalactosamine and serine or threonine residues) with a "bottle brush-like" structure: a long and flexible center protein chain presents regions densely coated with short glycans (the "brush"-like structure with approximately 3–10 nm in diameter and 50–200 nm in length), which alternate with folded "naked" hydrophobic regions that are rich in cysteine residues [35,36]. Once in aqueous media, mucins entangle and originate heterogeneous, complex jelly-like systems which are stabilized by intra- and intermolecular hydrogen bonding, electrostatic interactions and disulfide bridging between cysteine residues present in non-glycosylated regions [36]. The amount of disulfide bridging confers functionality to mucus and greatly influences its viscoelastic behavior. Membrane-associated mucins form a tightly packed layer of mucins called the glycocalyx, which is responsible for docking mucus to subjacent epithelia and may also play a role in mucoadhesion, particularly in the specific recognition of extracellular ligands [37].

In all cases, complete mucus fluids comprise a mixture of secreted mucus and other components resulting from the mucosal environment such as cells and their products (including debris), microbiota and microbiota-produced substances (*e.g.*, lactic acid in the vagina produced by lactobacilli [38]) or other fluids (*e.g.*, tissue exudates). Thus, besides water and mucin, mucus commonly contains variable amounts of DNA, plasma proteins, immunoglobulins (particularly secretory IgA), lysozyme, lactoferrin, lipids and polysaccharides depending on its anatomical localization, which may play important biological roles [39–41]. For example, a thin lipid layer may form on the outer surface of mucus at different sites and act as a barrier to the diffusion of gastric acid in the GIT or water evaporation in the tear film [35]. In the case of immunoglobulins, these biomolecules play an important role in aggregating and trapping pathogens in the mucus mesh, avoiding their spreading and promoting clearance [31]. Mucus from different sites possesses distinct properties as noted in Table 1. These variations are known to influence significantly mucoadhesion and should be considered when designing anatomical site-specific drug delivery systems. Differences in pH values immediately strike the eye, even for the same mucosal tissue depending on considered site (*e.g.*, in the stomach) or health status (*e.g.*, in the esophagus, the intestine or the vagina). These pH variations may clearly affect the conformation and charge of mucin. Sialic acid ($pK_a = 2.6$) strongly influences the electric charge of mucin (isoelectric point value ≈2–3 [42]); for example, nearly uncharged fluid may be expected in the apical layers of the stomach, while densely negatively charged mucus is present at the eye surface. These features may influence mucoadhesion, particularly when electrostatic forces are involved in mucin/polymer interaction, although the microstructure and bulk rheology of mucus seem to be relatively insensitive to the variation in proton concentration [43]. Further, mucus layer thickness, viscosity and turnover time are variable (Table 1) due to the dynamic structure of the mucin network, and can be altered depending on disease or physiological changes (*e.g.*, higher viscosity/lower clearance of sputum in cystic fibrosis patients, cervicovaginal dryness in menopausal women) and external stimuli (*e.g.*, increased respiratory mucus clearance upon contact with particulate matter, microstructural changes when in contact with different excipients or adhesive particles), with potential consequences in the behavior of mucoadhesive nanosystems [35,44–49].

3. Mucoadhesion phenomena at the nanoscale – mechanisms

3.1. Mucoadhesion basics

Bioadhesion is a particular case of adhesion and can be defined as the state where two materials, of which at least one is biological in nature, come in close contact and stay together for a substantial amount of time due to the establishment of interfacial bonding [89]. If the biological surface is a mucosa, then the phenomenon is usually referred to as mucoadhesion, and interfacial interactions may occur mainly with mucus but also with the epithelial cell lining.

Table 2
Different theories used to explain the adhesive interactions between mucoadhesive materials and mucus/mucosa as reviewed by Khutoryanskiy [90].

Theory	Short description
Electronic theory	Adhesion is established due to the electrostatic attraction between negatively charged mucin and positively charged materials
Adsorption theory	Adhesive interactions are related to the establishment of hydrogen and van der Waals bonding; hydrophobic effects and chemisorption may also contribute
Wetting theory	Adhesion is related with the ability of a mucoadhesive (when in liquid form) to spread over the mucus layer
Diffusion theory	Considers that adhesion is established by the interpenetration of macromolecular mucoadhesives (either polymeric or other) with mucin fibers, as driven by a concentration gradient differential
Fracture theory	Relates adhesion with the force required for interfacial detachment of two previously joint solid surfaces
Mechanical theory	Adhesion is dependent on the roughness of two different surfaces and the available area for interaction

Different materials may be used as mucoadhesives, though these are usually of polymeric or macromolecular nature.

Mucins are the main component of mucus fluids involved in mucoadhesion. Understanding the essential interactions between mucoadhesive materials and mucosal fluids is vital for the rationale development of mucoadhesive DDS. Different individual theories of adhesion have been suggested in order to explain mucoadhesion (Table 2) but only their combination can provide satisfactory understanding of the occurring phenomena [90]. In the particular case of polymeric mucoadhesives and after initial intimate contact between mucus and polymer, diffusion seems to play an essential role in the establishment of adhesive interactions; polymers diffuse and entangle with mucin fibers, while bonding is simultaneously established/disrupted. Bonding may be either covalent (*e.g.*, disulfide bridging with cysteine residues of mucin) or non-covalent (*e.g.*, electrostatic forces, hydrophobic interactions, hydrogen bonding, van der Waals bonding). The dynamic balance between diffusion, physical entanglement and adhesive/repulsive interactions leads to the consolidation of adhesion [91].

3.2. Nanosystems/mucus interactions

Although the basic principles by which intermolecular interactions occur are identical [92], size matters when considering mucoadhesion. Indeed, polymers can present substantially different mucoadhesive behavior either at bulk or nanoscale. The high surface-area-to-volume ratio of nanosystems is of the utmost importance as the interface available to establish bonding dramatically increases. This usually means that, upon the establishment of adhesive interactions between nanosystems and mucin, bonding endures for longer periods than in larger structures (Fig. 1) [19]. Counteracting repulsive forces, although present, are of lesser importance and cannot completely disrupt adhesive interactions [93]. Nanosystems can still be transported through the mucin matrix but remain mostly entrapped in it [46]. Besides influencing the available surface area, size also governs the ability of particles to fit in the low viscosity aqueous mesh spaces and channels formed within the mucin matrix that composes mucus. In one hand, mucoadhesive particles in the micrometric range tend to remain at the top layers of the mucus due to the inability to fit in these channels [94]; on the other, particles as large as 500 nm have been shown to penetrate the mucin mesh of cervicovaginal mucus, and adhere or diffuse within its interstices, as assessed by multiple-particle tracking (MPT) using video microscopy [33,46]. The previous upper limit size value may, however, not "fit" all types of mucus fluids present in the human body since mucin meshes vary according to pathological and non-pathological conditions [47]. For example, cystic fibrosis increases the micro-heterogeneity of sputum and only allows for smaller particles (around 200 nm or less) to be effectively transported, as assessed by MPT [95,96]. In contrast, Lieleg et al. [97] showed that particles as large as 1 μm can still be transported, even if presenting considerable hindrance, when mixed with reconstituted pig gastric mucus with low mucin concentration (0.25–0.5%). Thus, correct size adjustment seems to be a fundamental feature in the modulation of the mucoadhesive behavior of nanosystems in specific administration sites and pathophysiological conditions. However, the enhanced ability to penetrate mucus does not seem to be linear with size reduction. Again, MPT experiments using different sized mucus-penetrating polymeric nanoparticles (NPs), *i.e.*, polymeric nanosystems in which the surface has been modified in order to minimize adhesive interactions with mucin (see below), showed unexpected diffusion behaviors [46,98]. Mucus-inert NPs presenting diameters of 200–500 nm were able to show less hindrance to transport as compared to 100 nm counterparts. According to the authors of the study, 100 nm particles were capable to access smaller caliber channels within the mucus that often result in dead-end paths, thus reducing mobility of these particles due to physical entrapment rather than adhesive interactions with mucin. Thus, fine tuning of nanosystems size may be an interesting (and relatively simple, scalable and cost-viable) way to modulate mucoadhesion.

Another important aspect has to deal with surface chemistry and, in particular, surface charge. As mentioned above, the low isoelectric point of mucin determines its negative charge at most physiological pH values. This will then have an effect on the overall balance of adhesion strength as negatively-charged and positively-charged nanosystems will observe repulsive and attractive electrostatic forces, respectively, when in contact with mucus. Thus, positively-charged particles will present the potential to increase adhesion. This effect has been recently demonstrated for various polymeric NPs (size of approximately 200 nm) bearing different surface charge (negative or positive) by MPT using native pig GIT mucus (pH 6.5–7.5), and purified type II mucin reconstituted at different concentrations in different polyelectrolyte solutions

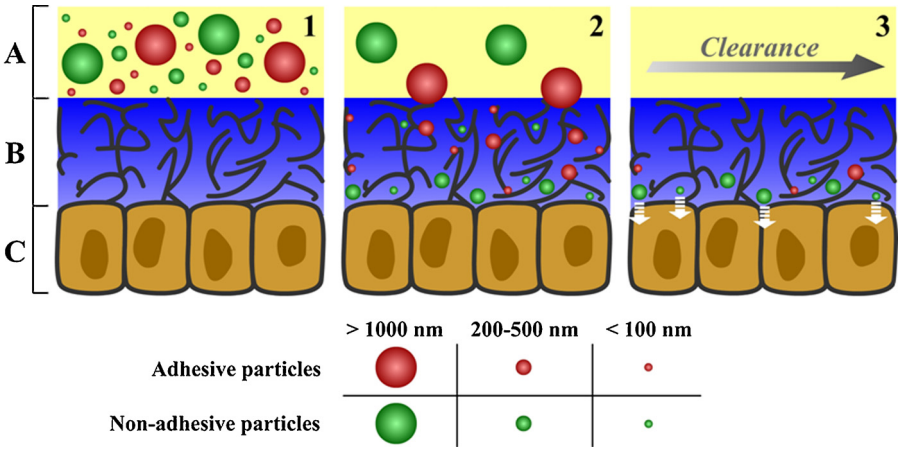


Fig. 1. Schematic representation of the interactions of several types of particles with the mucus layer. (1) After initial administration/arrival to the mucosal site, particles will interact and diffuse through the mucus differently according to their adhesive properties. (2) Larger particles (usually at the micrometric range) are not able to diffuse through the mucus layer due to steric hindrance, but can interact with the luminal layers in cases where adhesive bonding with mucin chains (in gray) can be established. As for smaller particles, these can diffuse through the mucus layer depending on adhesive properties: diffusion of adhesive NPs is slower as these will be retained particularly at the luminal/external layers of mucus due to interaction with mucin; in the case of non-adhesive NPs, systems with diameters around 200–500 nm can diffuse rapidly and reach the epithelial lining, while smaller ones (100 nm or less) experience decreased diffusion rates, presumably because of retention in "dead end" pockets of the mucin mesh. (3) Upon natural mechanisms of clearance, which are mostly felt at the luminal side of mucus, particles are progressively removed from the mucosal site while NPs that have reached the epithelial cell lining can further undergo cell uptake or tissue penetration (represented as white dashed arrows. Legend: A – mucosal tissue lumen/external environment; B – mucus layer; C – epithelial cell lining [19], Copyright 2011.
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with pH ranging from 4.2 to 7.4 [87,99]. Besides charge, chemical moieties present at the surface of nanosystems also impact on the mucoadhesive potential. In general, promoting adhesive interactions with mucus by any of the above mentioned mechanisms will increase the mucoadhesive potential of nanosystems; of particular interest, and the main topic of this review, is of course the presence of polymers at the surface of drug nanocarriers.

3.3. Modulation and assessment of the mucoadhesion of nanosystems

As detailed above, surface chemistry, charge and size of nanosystems determine their mucoadhesive behavior. These properties are tunable in order to maximize or minimize interactions with mucus fluids present at different mucosae, as summarized in Fig. 2 [100]. When mucoadhesive systems are required, this can be simply achieved by using mucoadhesive polymers as matrix-forming materials, alone or in mixtures. However, not all the available mucoadhesive polymers can readily or easily be used to produce NPs [101] or, for instance, other non-polymeric systems may be preferable (*e.g.*, lipid-based nanosystems). In the last case, surface modification can be an alternative, either by attaching mucoadhesive polymers on preformed nanosystems (covalently or by simple adsorption) or by conjugating polymers with other matrix-forming materials. Another important aspect is the charge at the surface; positively-charged systems are preferred in order to maximize mucoadhesion. Chitosan (CS)-based NPs, in particular, are often considered as the typical example of highly mucoadhesive nanosystem [102–104]. Also, hydrophobic nanosystems may possess high ability to establish adhesive interactions with hydrophobic domains of mucin, namely by promoting hydrophobic bonding [105]. In contrast with the previous, mucus-inert nanosystems that avoid interaction with mucus may also be desirable [100]. Thus, mucus-penetrating NPs can be generally obtained by conferring a hydrophilic uncharged surface. The group of Justin Hanes at Johns Hopkins University (Baltimore, MD, USA) demonstrated this by modifying the surface of different polymeric NPs (100–500 nm) with a dense layer of low molecular weight poly(ethylene glycol) (PEG; 2–10 kg/mol) [32]. For example, MPT experiments showed that 200 nm PEG-modified NPs diffused in cervicovaginal mucus at rates near the ones predicted for the same sized nanospheres in water (up to around one-log hindrance), while non-PEGylated ones were nearly immobile (diffusion rates reduced by at least 3-log) [46,106,107]. The hydrophilic and nonionic nature of PEG avoids the establishment of hydrophobic and ionic bonding, respectively, while the short chain of the polymer diminishes mucoadhesive entanglement with mucin fibers [98,108]. These observations for densely, short chain PEG-modified nanosystems have also been confirmed by other groups [109–111]. For instance, when tested *in vivo* and after vaginal delivery to mice, densely PEG-modified NPs were shown able to readily distribute throughout the cervicovaginal tract and provide enhanced drug delivery to the underlying mucosa due to their ability to tackle the mucus barrier [112]. Moreover, this strategy was proved advantageous in a mice model of vaginal herpes simplex virus type 2 (HSV-2) as evidenced for PEGylated mucus-inert acyclovir NPs [113].

As for size, depending on the properties of mucus present at different anatomical sites, particular size ranges

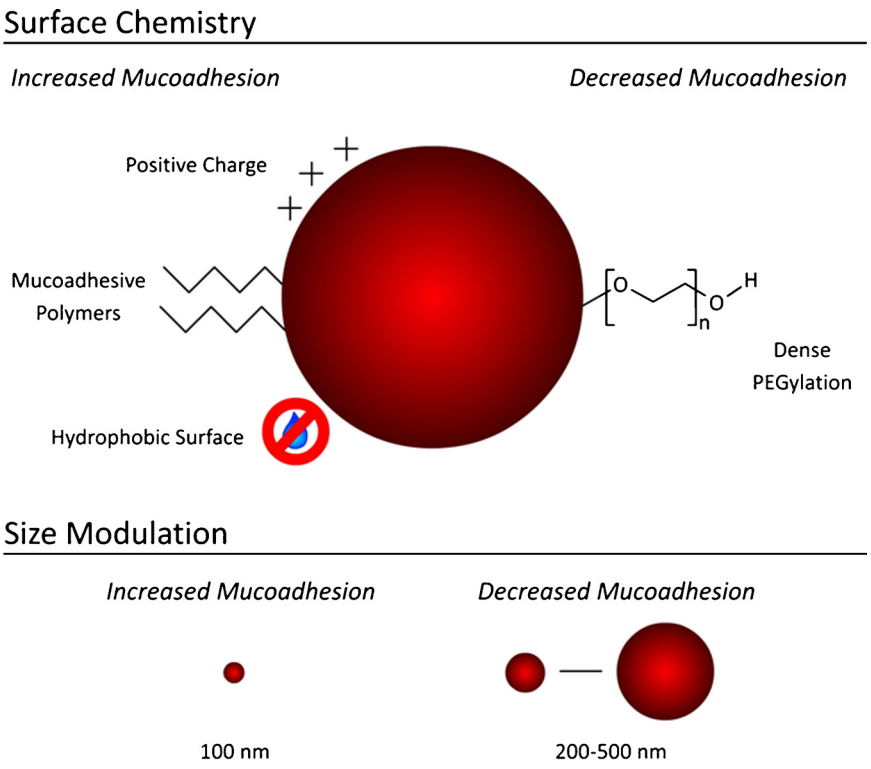


Fig. 2. Different strategies used for engineering the mucoadhesive behavior of nanosystems. Size ranges presented have been determined for cervicovaginal mucus and may not be applicable to other mucus fluids (see text for details) [100], Copyright 2011. Reproduced with permission from John Wiley & Sons.

may be preferable either to increase or decrease diffusion along the aqueous channels formed by the mucin matrix. In this case, adhesion is based on physical hindrance rather than on interfacial interaction, but it still presents substantial influence on the overall mucoadhesiveness of the nanosystems. One important aspect to keep in mind is that the structure of mucus fluids is dynamic and can be highly variable on the surrounding conditions. Besides pH, the possible presence of other substances (*e.g.*, electrolytes, chemicals, pharmaceutical excipients) may impact the arrangement of mucin fibers and, thus, the mucoadhesive behavior of NPs [45,48,114]. Moreover, the simple establishment of adhesive interactions of NPs with mucin can impact on the structure and the viscoelastic properties of mucus [115,116].

Different experimental methods can be used for the characterization of the mucoadhesive potential of nanosystems. These can be classified as indirect or direct. The former are based on the evaluation and balance of contributing and detrimental interactions between nanosystems and mucins or other mucosal components (tissue or mucus), while the latter are performed *in vivo* (animal or humans) or in close proximity to the *in vivo* situation (*ex vivo* settings). A summary of different direct and indirect methods is presented in Table 3. The detailed description of each is out of the scope of this manuscript but readers are referred to a recent review by the authors on the subject [19].

4. Mucoadhesive polymers

4.1. Natural polymers

4.1.1. Alginate

Alginates (ALG) are a series of natural unbranched polyanionic polysaccharides that combine β-D-mannuronic acid (M) and α-L-guluronic acid (G) linked by $1 \rightarrow 4$ linkages and arranged in MMMMM and GGGGG homo-sequences inter-dispersed with MGMGMG hetero-sequences [117,118]. Molecular weight ranges between 32 and 400 kg/mol, and different G/M compositions, lengths of each homo-segment and molecular arrangements that can be precisely established by ^{1}H NMR [119,120] have given place to more than 200 ALG types currently available in the market [121]. Even though ALG could be isolated from algal and bacterial sources, the commercially available derivatives are mainly extracted from brown algae such as *Laminaria hyperborea, Ascophyllum nodosum* and *Macrocystis pyrifera* [119,122,123]. The relative G/M composition depends on the source, the season of harvest and the age of the plant used [124]. It is worth stressing that the chemical composition of algal ALG is less reproducible than that of the bacterial one.

The physicochemical and rheological properties of ALG and its aqueous solutions are intimately related to the G/M compositional ratio, the molecular weight and the polymer concentration. Aqueous ALG solutions can undergo sol–gel transitions upon ionotropic crosslinking through chelation

Table 3
Different techniques for the evaluation of mucoadhesion of nanosystems and comparison of their key features.

Methods for measuring mucoadhesion	Insight on mechanism	Dynamic/real-time measurement	*In vivo* relevance	Feasibility	Cost
Indirect					
Mucin particle method	Low	No	Low	High	Low
Microgravimetric methods	Medium	Yes	Low	Medium	Medium
Atomic force microscopy	High	Yes	Low	Low	High
Optical techniques	Medium	Yes	Low/medium	Medium	High
Diffusion/particle tracking methods	High	Yes	Medium	High	Medium
Direct					
Cytoadhesion methods	Medium	Optional	Medium	Medium	Medium
Ex vivo methods	Low	Optional	Medium	Medium	Medium
In vivo administration/*ex vivo* analysis	Low	No	High	Medium	Medium
In vivo imaging	Low	Yes	High	Low	High

Adapted from [19] with permission of Informa Healthcare (Copyright 2011).

of Ca^{2+} or other divalent ions by the pendant carboxylic groups of the G blocks in an "egg-box" model structure (Table 4) [119]. The mechanical and the physical stability of ALG gels depend on the G content; the greater the G content, the more rigid and brittle the matrix. The process can be reverted in presence of ion sequestrants such as ethylenediaminetetraacetic acid (EDTA) or sodium citrate and ALG gels tend to be eroded under more neutral and basic pH values [125]. From a regulatory point of view, the US FDA recognizes ALG as "Generally Recognized As Safe" (GRAS), a designation that applies to substances that are accepted as safe for alimentary use by qualified experts [126]. GRAS excipients are listed in the Code of Federal Regulations, Title 21 (21 CFR), parts 182 and 184.

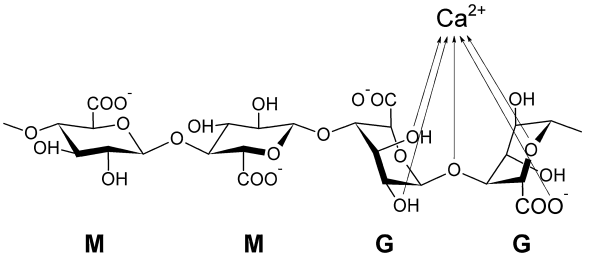
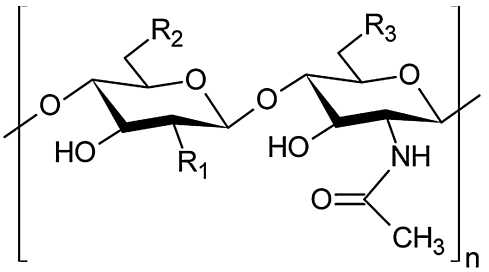
ALG has been shown to be bio- and mucoadhesive, biocompatible and non-irritant, thus finding commercial application in the production of wound dressings with different features such as exudates absorption, hemostasis and moisture conservation that favor epithelialization and wound healing [127–130]. The pro-thrombotic coagulation mediated by ALG has been also shown [131,132]. Still, there is some controversy regarding specific adverse effects like immunogenicity associated with ALG [133,134] that could likely stem from the quality and level of purity of the biomaterial employed in the different studies [135] and from an intrinsic immunogenic property; *e.g.*, traces of heavy metals, endotoxins, proteins, and polyphenolic compounds could remain in the final product and lead to undesired effects. When purified in a multi-step extraction methodology, no foreign body response was apparent in animals upon intramuscular implantation [136]. Moreover, owing to its gelation capacity, ALG has been exploited in cell encapsulation and immunoisolation [137,138], and the encapsulation and sustained release of a broad spectrum of drugs [117,119]. ALG is being employed in different pharmaceutical applications as thickening and gel forming agent, especially for oral administration. At the same time, the use in depot DDS is growing [139]. Water-soluble drugs are mainly released by diffusion and poorly water soluble drugs by matrix erosion. The release of small molecules is fast due to the fact that ALG generate nanogels with a pore diameter of approximately 5 nm [131,133]. However, modifications can be pursued to physically or chemically bind the drug to the network and, by doing so, to sustain the release over time. An additional interesting feature of ALG is that dry systems are mucoadhesive, thus extending the residence time and the release in different mucosal tissues [140–145]. Due to the high chemical functionality (two –OH and one –COOH per repeating unit), the chemical modification of the side chain of ALG has been extensively explored to increase the solubility in aprotic solvents [118,146] and modify other physicochemical properties [147,148], to attain biomimetic [149] and amphiphilic features [150] and to conjugate cell signaling ligands [137,151]. For an extensive and thorough overview of the chemical modification of ALG, the readers are referred to the review of Pawar and Edgar [118].

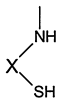
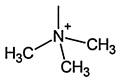
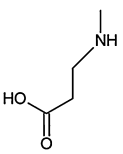
4.1.2. Chitosan and its derivatives

CS is a linear polysaccharide of random β-(1 → 4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit) (Table 4) extracted from shrimps and other crustacean shells as its water insoluble precursor chitin and generated by deacetylation with sodium hydroxide [152]. The extent of deacetylation can vary, giving place to CS with different acetylation extents and properties. General advantageous features of CS include good blood, cell and biocompatibility and biodegradability [153–155]. Also, different studies reported on the antimicrobial [156] and antioxidant [157,158] activity of CS.

CS is a polycation, thus its aqueous solubility is pH-dependent and only possible below a pH value of approximately 5.8 due to the progressive protonation of pendant amine moieties. Due to the ability to establish ionic, hydrogen and hydrophobic bonds with the negatively-charged mucin, CS has emerged as a fundamental mucoadhesive biomaterial [159] for a broad variety of administration routes such as oral [160,161], ocular [162], nasal [163], inhalatory [164] and topical [165]. In addition, CS has been chemically modified to fine tune its properties toward the encapsulation and delivery of different drugs, genes and proteins [164]. Moreover, the natural mucoadhesiveness can be improved by the incorporation of chemical groups that increase the interaction with components of the mucus. One of the most effective strategies is grafting of thiol moieties to the chain of CS and other multifunctional polymers [166]; the process has been coined thiolation. For example, the mucoadhesiveness of thioglycolic acid/glycol CS NPs was compared to that of

Table 4
Structure of the most relevant mucoadhesive polymers.

Polymer	Structure
Alginate	
Chitosan and selected derivatives	

Polymer	R_1	R_2	R_3
Chitosan			
Thiolated chitosan			
N-trimethyl chitosan			
N-carboxymethyl chitosan			
O-carboxymethyl chitosan			

Guar gum

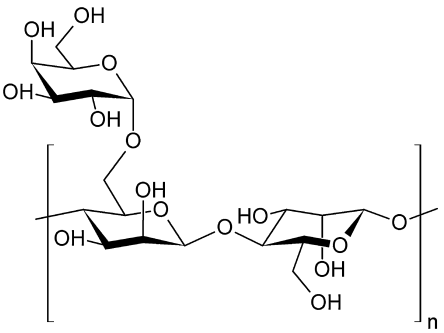


Table 4 (*Coninued*)

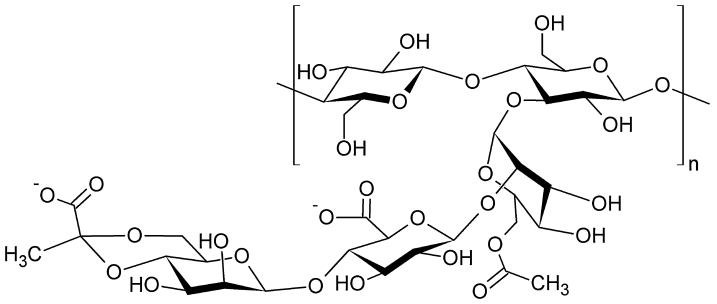
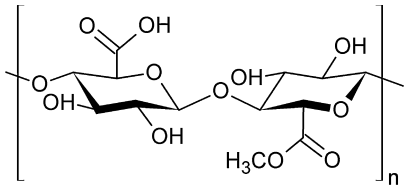
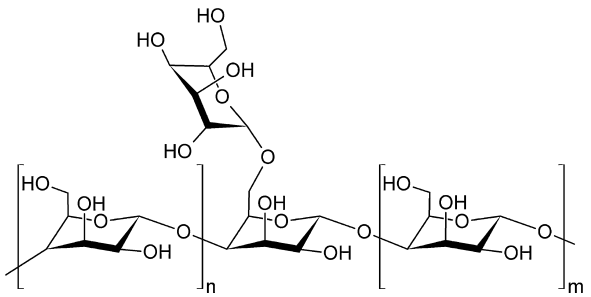
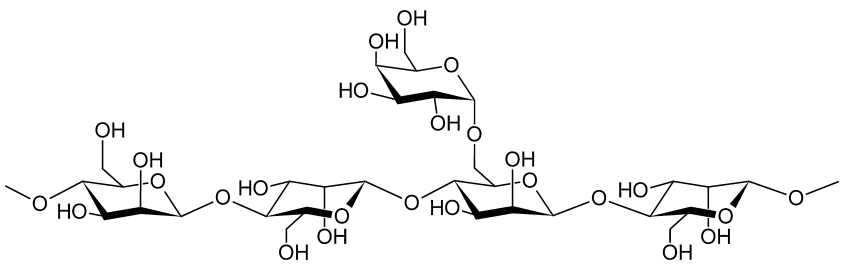
Polymer	Structure
Xanthan gum	
Pectin	
Galactomannan	
Glucomannan	

Table 4 (*Coninued*)

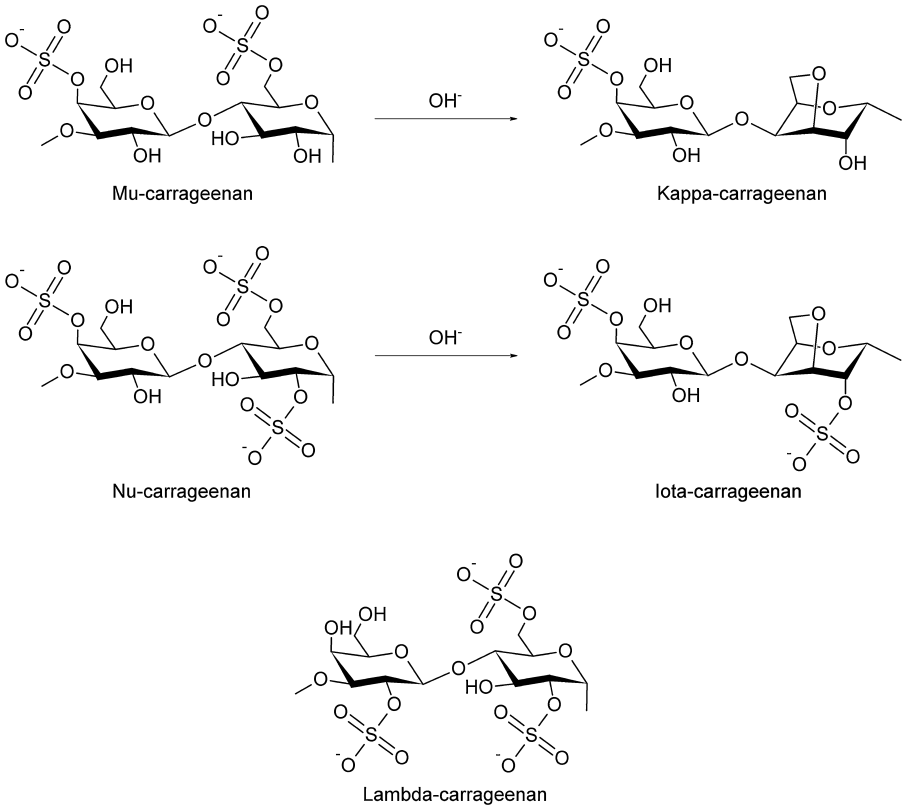
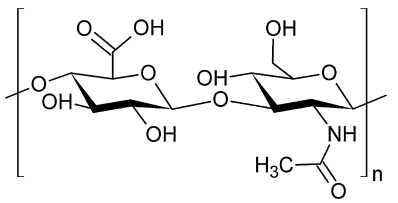
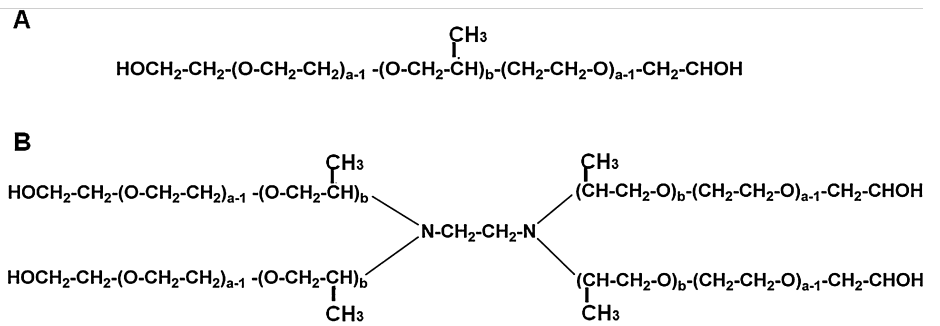
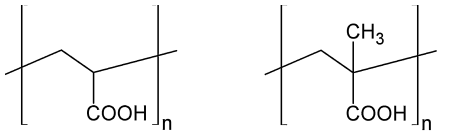
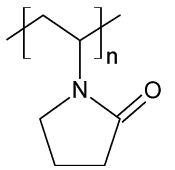
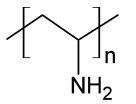
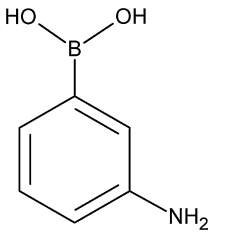
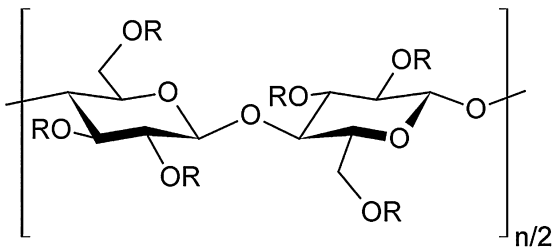
Carrageenans	
Hyaluronic acid	
Poly(ethylene oxide)-*b*-poly(propylene oxide) copolymers	

Table 4 (*Coninued*)

Poly(acrylate)s, poly(methacrylate)s	 **A** **B** A: Poly(acrylic acid) B: Poly(methacrylic acid)
Poly(vinyl pyrrolidone)	
Poly(vinyl amine)	
3-Aminophenylboronic acid	
Cellulose and selected derivatives	

Polymer	R groups
Cellulose	H
Methylcellulose	H, CH_3
Ethylcellulose	H, CH_2CH_3
Hydroxyethyl cellulose	H, CH_2CH_2OH
Hydroxypropyl cellulose	H, $CH_2CH(OH)CH_3$
Hydroxypropyl methylcellulose	H, CH_3, $CH_2CH(OH)CH_3$
Carboxymethyl cellulose	H, CH_2COOH

non-thiolated counterparts for intratracheal administration in rats [167]. The increased pulmonary mucoadhesion correlated well with the increase of the bioavailability of calcitonin. On the other hand, the mucoadhesive properties of CS and its chemically-modified derivatives could also represent a drawback due to the more limited penetration of these nanocarriers across mucus. CS is also capable of transiently disrupt cell tight junctions, enhancing the absorption of drugs by the paracellular route upon oral and also inhalatory administration [168]. Yamamoto and collaborators compared the pulmonary absorption of carboxyfluorescein and fluorescein isothiocyanate–dextran with molecular weight between 4 and 70 kg/mol in the presence or absence of CS employing a guinea pig model [169]. CS significantly increased the permeation for all model compounds, the enhancement being more

remarkable for higher molecular weight isothiocyanate–dextran owing to the fact that it is mainly absorbed by the paracellular route. However, it should be stressed that the toxicity of CS and its derivatives depends on the administration route; *e.g.*, Calu-3 cells exposed to CS microparticles displayed the release of inflammatory cytokines (IL-2 and IL-8) [170]. Similar observations were reported *in vivo* after the intratracheal administration of CS and two different *N*-trimethylated chitosans (TMC), where neutrophil infiltration and structural damage in the lung parenchyma were observed [171]. However, authors attributed this phenomenon to the physical obstruction of the bronchioles and not to the inherent toxicity of CS. These aspects should be further investigated to support the usefulness of this polysaccharide in specific more sensitive administration routes. In fact, in other studies, intratracheal CS NPs and microparticles provoked a similar inflammatory response [172,173].

4.1.3. Guar gum, xanthan gum and pectin

Guar gum is a non-ionic polysaccharide classified as a galactomannan and it comprises a linear chain of β-(1–4)-D-mannose to which side residues of α-D-galactose are attached by 1 → 6 linkages (Table 4). The typical *D*-mannose/*D*-galactose ratio is 1.4:1 to 2:1 and, although precise molecular weight cannot be defined, it has been estimated around 200–300 kg/mol [174]. Guar gum is obtained from the ground endosperms of guar beans (*Cyamopsis tetragonolobus*). It is approved in oral and topical pharmaceuticals, featuring monographs in the US Pharmacopeia and European Pharmacopoeia [175]. It is also considered a safe excipient and food additive, being listed as a GRAS product by the US FDA [176]. Guar gum is mostly used in pharmaceutical products due to its ability to swell and originate viscous colloidal dispersions or sols upon dispersion in water, which is related to its mucoadhesive properties [177]. In addition, since it is degraded by bacterial enzymes present in the colon, it has been particularly investigated over the last years for colonic drug targeting upon oral administration [178].

When compared to other commonly used mucoadhesive polymers such as hydroxypropyl cellulose (HPC), carboxymethyl cellulose (CMC) or Carbopol® 971P, guar gum performed well when used in both dry or gel state, presenting comparable adhesive properties [179]. However, other studies indicated that it may lack the strong mucoadhesiveness of CS [180]. Thus, guar gum has been recurrently used in association with other polymeric and non-polymeric compounds for obtaining mucoadhesive pharmaceutical products [181–183]. Guar gum is also a versatile polymer because it can undergo chemical modifications (for an excellent review see reference [184]). Changes in its structure can impact mucoadhesive properties. For instance, hydroxyl groups can be replaced by trimethylammonium ones conferring positive charge to the molecule. This change has been shown helpful in mildly increasing the mucoadhesive properties of buccal films comprising an inter-polymer complex with negatively-charged xanthan gum [185]. In another study, Lu et al. reported on the interaction of negatively-charged hydroxypropyl guar–borate complexes with mucin. These investigators found that these complexes did not bind to mucin under eye physiological pH values (7.3–7.8), presumably due to electrostatic repulsion [186].

Alongside guar gum, xanthan gum has been widely used in the pharmaceutical and food industries, mainly due to its viscosity enhancing properties [187]. This natural high molecular weight (2×10^3 to 20×10^3 kg/mol) polysaccharide is produced by the bacterium *Xanthomonas campestris* and comprises *D*-glucose, *D*-mannose and *D*-glucuronic acid in a 2:2:1 ratio [187,188]. It presents a specific structure: the backbone chain is composed of β-(1–4)-D-glucose, which presents a trisaccharide (β-(1–3)-D-mannose-α-(1–2)-(glucuronic acid)-β-(1–4)-mannose) at the *O*-3 position of alternating anhydroglucose residues (Table 4). The terminal mannose unit is substituted by pyruvate at positions *C*-4 or *C*-6 of roughly 50% of the trisaccharides, while the non-terminal mannose usually presents an acetyl group at the *C*-6 position. The polymer is arranged as a single, double or triple helix in solution and presents negative charge due to the presence of carboxylic acid groups [187,188]. Xanthan gum presents monographs in the US Pharmacopeia and European Pharmacopoeia and is approved in the US and European Union as a pharmaceutical ingredient (oral and topical). It is also approved as a food additive in the US (GRAS status) and Europe [174].

Xanthan gum has been used for developing mucoadhesive products particularly for ophthalmic use [189]. However, the mucoadhesive properties of xanthan gum have been described as low to mild as it only interacts moderately with mucin [190]. This low interaction may be explained, at least partially, by the high molecular weight of xanthan gum, which limits interpenetration, and electrostatic repulsion between negatively-charged chains of the polymer and mucin. Even so, an early study found that the interaction ability of xanthan gum with mucin was similar to that of Carbopol® 934P but higher than for ALG, hydroxypropyl cellulose (HPC) and poly(methyl vinyl ether-co-maleic anhydride) [191]. In a recent work, xanthan gum presented higher mucoadhesive potential in the solid state as compared to guar gum but lower than glycol CS or Carbopol® 934, when tested for the buccal drug delivery of nicotine [180]. Thus, its use in combination with other mucoadhesives is frequent [192,193], mainly due to its ability to modulate drug release and increase viscosity. An interesting feature of xanthan gum is the synergistic effect on viscosity when mixed with galactomannans such as guar gum and its derivatives [194]. The presence of different reactive groups on the molecular structure of xanthan gum makes it an excellent candidate for chemical modification [184]. As in the case of guar gum, the impact on mucoadhesion may be of relevance but the issue has not been, to our best knowledge, effectively addressed.

Pectin is another natural polysaccharide commonly used with pharmaceutical and food purposes, mainly due to its gelling and thickening ability [195]. Its use has been proposed for developing controlled-release, gastro-retentive and colon-targeted formulations [196]. In particular, the polymer is completely fermented in the colon by local microbiota [197]. Pectin is a complex anionic polysaccharide in which its simpler and most abundant form, homogalacturonan, comprises a linear

α-(1–4)-D-galacturonic acid chain, being partially esterified with methoxy groups at the *C*-6 carboxyl and eventually acetylated at the *O*-2 and *O*-3 positions (Table 4). In more complex forms, the main homogalacturonan chain displays different pendant monosaccharides such as *L*-rhamnose (rhamnogalacturonan II), *D*-xylose (xylogalacturonan) or *D*-apiose (apiogalacturonan). Also, the main chain monomer can be composed of (4)-α-D-galacturonic acid-(1–2)-α-L-rhamnose-(1), usually termed rhamnogalacturonan I [198]. Pectin is present in the cell wall of all higher and most terrestrial plants although available pectins are mostly obtained from citrus peel or apple pomace [195]. Its molecular weight is variable and estimated in the range of 30–150 kg/mol. Pectin is regarded as non-toxic and has been granted GRAS status in the US and is approved in Europe as a food additive. It presents a monograph in the US Pharmacopeia.

Pectin is only mildly mucoadhesive and its mucoadhesive potential and mechanisms have been well characterized using different techniques. Adsorption to mucin due to hydrogen bonding seems to be the main mechanism of mucoadhesion; also, electrostatic repulsion in aqueous media may contribute to the uncoiling of polymer chain, thus facilitating interpenetration with mucin [199,200]. Different characteristics of pectin may influence mucoadhesive forces, including molecular weight, chain flexibility and the abundance of methoxy groups [201–204]. In this last case, higher degree of esterification seems to be associated with increased mucoadhesiveness [205], even if conflicting results have also been reported [206,207]. The pH and presence of electrolytes further influences the behavior of pectin. Chemical modification of the polymer, namely deamidation (commercially available pectin can be treated with ammonia thus possessing amide groups; deamination exposes amine and carboxylic acid groups, and makes them available for establishing mucoadhesive bonding) [208] and thiolation (*i.e.*, modification with thiol groups that can establish disulfide bonding with cysteine groups of mucin) [209], can be performed to increase adhesive interactions. Also, low methoxylated pectin seems to be of interest concerning its mucoadhesive performance [210]. When compared to other polymers using high-resolution ultrasonic spectroscopy, highly-methoxylated pectin presented relatively lower ability to interact with mucin in solution than hydroxypropyl methylcellulose (HPMC; *e.g.*, Methocel® K4M Premium EP) and Carbopol® 974P [197]. In another study, pectin was also shown to be less mucoadhesive than CS, Carbopol® 934, Carbopol® 940 and polycarbophil when used for the formulation of buccal tablets [201].

4.1.4. Galactomannan and glucomannan

Galactomannans (GalM) are neutral vegetal polysaccharides stored in the endosperm of different leguminosae and formed by a 1 → 4-linked β-D-mannopyranose backbone with pendant residues of 1 → 6-linked α-D-galactopyranose (Table 4) [211,212]. GalM can be obtained from different gums and, depending on the source, the mannose-to-galactose molar ratio changes from 1:1 for fenugreek to 2:1, 3:1 and 4:1 for guar, tara and locust bean gums [213–216]. In addition, the molecular weight can be affected by the isolation method that, depending on the strength of the conditions, could result in chain scission [217]. GalM have found broad application in food industry as stabilizer [218] and more recently in the development of biomedical products [214].

Glucomannan (GluM), also known as Konjac fiber, is a water-soluble mainly unbranched polysaccharide of β-(1 → 4)-linked *D*-mannose and *D*-glucose in a ratio of 1.6:1 [219] (Table 4). The degree of branching is usually below 10%. It is obtained from *Amorphophallus konjac* and has found application as dietary supplement and food additive [220]. Regardless of its potential, the US FDA has not approved it for pharmaceutical use, while Health Canada has authorized it for appetite and cholesterol reduction. Some attempts to develop GluM-based DDS have been pursued, most of them combined with other polysaccharides [221]. Due to its mucoadhesiveness, it has been used to develop a protecting and healing device for the treatment of esophagus disorders [222] and in wound healing [223]. The gelation profile of GluM can be fine tuned by means of different extents of acetylation [224,225]. The group of Maria Jose Alonso (University of Santiago de Compostela, Spain) has recently reviewed the potential of GluM in different (bio)pharmaceutical applications [226]. An appealing molecular feature of these polysaccharides is that they display galactose and/or mannose, sugars that are specifically recognized by transmembrane receptors known as lectin-like receptors (LLRs) that are highly expressed in different cell types such as macrophages, dendritic cells, Langerhans cells and hepatocytes [227,228]. Thus, the modification of drug nanocarriers with mannose and galactose residues has been extensively explored to actively target drugs to LLR-expressing cells [226,227,229–237]. For example, our group has developed polymeric micelles surface-modified with hydrolyzed GalM for the active targeting of rifampicin to macrophages *in vitro* [238]. Hydrolysis was meant to reduce its molecular weight and viscosity in water solution that made the processing very difficult. To exploit this property, Dong et al. modified GluM with *N,N′*-carbonyldiimidazole/ethylenediamine to confer the polymer DNA binding capacity and targeted an oligodeoxynucleotide to macrophages [239].

The research of GalM and GluM in the development of conventional and modified DDS for oral administration has been relatively profuse and usually in combination with other polysaccharides gum [240–242]. Jian et al. produced GalM/xanthan gum matrix tablets for the controlled delivery of theophylline over 24 h (Fig. 3) [241]. The combination enabled the fine control of the swelling and the thickness of the gel-like and the infiltrated layer formed that could affect the mucoadhesiveness. Conversely, the release kinetics remained almost unchanged. These findings opposed a previous report by Vendruscolo et al. where the same polysaccharides combination was used to adjust the release kinetics of the same drug and the contribution of Fickian diffusion to the mechanism. It is worth mentioning that the GalM source was different, what could have changed its performance [243].

The source and inter-batch variation of natural polymers represents a disadvantage that has been overcome in some cases by developing biotechnology production

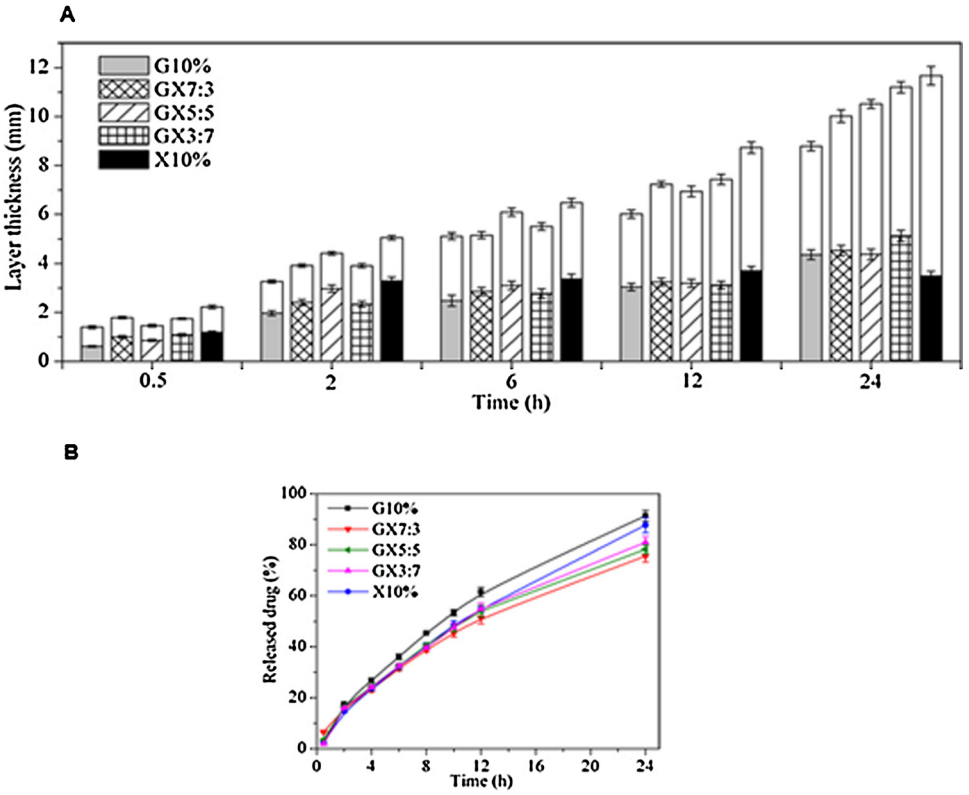


Fig. 3. (A) Thickness of the gel-like layer (upper bar) and the infiltrated layer (lower bar) after swelling at different time points. (B) Release profile of theophylline from tablets with GalM and xanthan gum as sustained release biomaterials [241], Copyright 2012.
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methods. Otherwise, comprehensive characterization protocols and strict approval specifications are required to ensure the reproducible performance of the product that contains them. Others assessed its potential as binder, coating, and tablet disintegrant [243–255]. Burke and coworkers designed a GalM-based DDS for specific colonic delivery that undergoes hydrolysis by β-mannanase, an enzyme that becomes active under the pH conditions of the colon and degrades the matrix to release the model drug [256]. More recently, Liu et al. developed a DDS made of GluM and HPMC for the pulsatile colonic release of 5-aminosalicylic acid [257]. Plasma-time profiles showed that the C_{max} is attained after 7 h (Fig. 4) [257].

Other combinations with CS [258], xanthan [259–261], gelatin [258] and poly(acrylic acid) (PAA) [262] have been studied. At the same time, applications neither especially emphasized nor assessed the mucoadhesive properties of GalM and GluM.

4.1.5. *Carrageenan κ type II*

Carragenans are linear polysaccharides that display disaccharide repeating units of 1→3-linked α-D-galactopyranose (G-units) and 1→4-linked β-D-galactopyranose (D-units) [263,264]. They differ from agars in that D-units are in the D-form instead of the L form. They are extracted from red seaweeds and named with Greek letters according to the number of sulfate groups per dimer [265]. A total of 15 carrageenan structures have been identified, being kappa (κ) with one sulfate group, iota (ι) and mu (μ) with two sulfate groups, and lambda (λ) and nu (ν) with three sulfate groups per dimer the most relevant (Table 4). Some seaweeds contain relatively pure carrageenan derivatives. For example, *Euchema cottonii* contains mainly κ and μ and *Euchema spinosum* ι. While κ and ι varieties form stable physical gels, κ-carrageenan

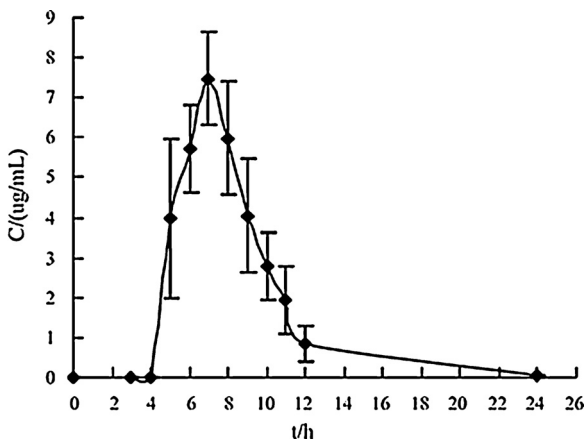

Fig. 4. Plasma concentration–time profile of 3 beagle dogs after oral administration of a 5-aminosalicylic acid pulsatile capsule [257], Copyright 2012.
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hydrogels exhibit both pH- and temperature-responsiveness. Conversely, γ carrageenan is thickener/viscosity builder. Carrageenans are used in a variety of commercial applications as gelling, thickening, and stabilizing agents, especially in food products [266]. Commercial carrageenans have an average molecular weight between 400 and 600 kg/mol with a minimum of 100 kg/mol; smaller molecular weight derivatives were reported to produce cecal and colonic ulceration. Among the different types, carrageenan κ type II is the derivative most extensively used as matrix for biomedical applications [265,267,268]. Furthermore, a few works reported on the biological activity of different derivatives, such as anticoagulant [269,270], antibacterial [271,272], antiviral [273,274], anti-oxidant [269] and in the prevention of sexually transmitted diseases [274–276]. A remarkable adverse effect of parenteral carrageenan is the pro-inflammatory activity that provokes edema and pleurisy [270]. On the other hand, its employment as mucoadhesive biomaterial remains undercapitalized.

4.1.6. Hyaluronic acid and other glycosaminoglycans

Hyaluronic acid is a glycosaminoglycan composed by disaccharide units of *D*-glucuronic acid and *N*-acetyl-D-glucosamine connected alternatively by β-(1 → 3) and β-(1 → 4) bonds (Table 4) [277]. While traditionally extracted from rooster combs, hyaluronic acid is nowadays produced essentially by means of fermentation as mediated by *Streptococcus* sp. [278]. It differs from other glycosaminoglycans (*e.g.*, keratan sulfate, chondroitin sulfate/dermatan sulfate, and heparan sulfate/heparin) essentially because of the absence of sulfate moieties. This natural unbranched anionic polymer (pK_a = 2.9; molecular weight = 10–10^4 kg/mol) plays an important role in the composition of the extracellular matrix and synovial fluids of mammals, being biocompatible and biodegradable by hyaluronidases [277]. Also, hyaluronic acid is degraded at either pH < 5 or pH > 10 in a time-dependent fashion. Its viscosity in solution is highly dependent on the shear rate, making it an excellent lubricant [279]. The sodium salt of this anionic polymer, sodium hyaluronate (hyaluronan), is most commonly used in the manufacture of medicines and medical devices and has been approved by the US FDA for either parenteral or topical use. Hyaluronic acid/sodium hyaluronate is also useful for the management of arthritis, being implanted by intra-articular injection; other applications include tissue regeneration and repair in ophthalmology and cosmetics [280,281].

The mucoadhesive properties of hyaluronic acid have been mainly attributed to its ability to establish hydrogen bonding and electronic interactions with mucin. Indeed, a recent study using NMR demonstrated that hyaluronic acid (950 kg/mol) can interact with mucin [282]. However, a previous investigation using a resonant mirror biosensor indicated scarce interaction of sodium hyaluronate (620–1150 kg/mol) with bovine submaxillary mucin in the pH range of 4.0–8.2 [283]. This fact may be related to the low pK_a of the polymer (≈3.2); at the considered pH values, the molecule is completely ionized, which can lead to repulsive electronic interaction with also negatively charge mucin (isoelectric point around 2–3 [42]). Furthermore, a recent study using the surface plasmon resonance technique indicated that interpenetration and physical interlocking with mucin chains, rather than chemical interaction, is the most probable mechanism involved in the mucoadhesive behavior of this polymer [284].

When compared to other polymers, highly polymerized sodium hyaluronate tablets presented *ex vivo* adhesion time values similar to that of polycarbophil, Carbopol® 971, and sodium CMC (NaCMC) [285]; the assay employed intestinal pig mucosa at pH 6.8 in a rotating cylinder and the parameter measured was the time required for the detachment of the tablet. However, mucoadhesion was reduced by roughly 3-times or even more when the tested polymer was pretreated at pH 3 before being freeze-dried or precipitated. In this last case, pH-dependent degradation may have played a role. Molecular weight of hyaluronic acid may also have an impact on its adhesive properties as found by Sandri et al. [286]. In their investigation, shorter chain hyaluronic acid in the range of 2×10^2–2×10^3 kg/mol appeared to result in improved mucoadhesion to buccal, vaginal and rat intestinal (jejunum) mucosae. The authors correlated these observations with the optimal length of hyaluronic acid to interpenetrate mucin chains and establish mucoadhesive bondings.

Modification of hyaluronic acid has further been pursued in order to enhance mucoadhesiveness. In particular, the synthesis of a thiolated hyaluronic acid–cysteine ethyl ester conjugate was shown effective in achieving over 6.5-fold increase with respect to unmodified hyaluronic acid (MW = 1.3×10^3 kg/mol), as assessed by measuring the *ex vivo* adhesion time of tablets to pig intestinal mucosa [287]. In another study, Li et al. modified hyaluronic acid with *L*-cysteine and showed that the thiolated polymer presented 2–5-times higher adhesion to rat small intestinal mucosa than native hyaluronic acid, when tested in a tensile test [288]. Moreover, there was a clear positive correlation between the degree of thiol substitution and the increase of the adhesion force. In addition, physical mixtures of hyaluronic acid with tamarind seed polysaccharide have been shown synergistic in increasing mucoadhesion, presumably due to the establishment of nonspecific interpolymer interactions [289].

4.1.7. Gelatin

Gelatin is a water-soluble linear polypeptide [290] obtained by dissolution and partial hydrolysis of collagen in acid or basic medium under heating from bones, skin and other connective tissue of different animals [291]. The molecular weight range is very broad and could be between 3 and 100 kg/mol.

It has been used in food and pharmaceutical industries for the production of coatings, thermo-responsive gels and capsules. Even though a repeating unit cannot be identified in gelatin and its polymeric nature could be questioned, this protein can be regarded as a typical polymer. Owing to its profuse pharmaceutical use, we included it in this article. The amino acid sequence and composition has been investigated over the years by different groups [292]. Gelatin displays 18 amino acids, alanine and glycine comprising one third to one half of the total residues, based on the type of hydrolysis [293]. Approximately one quarter of the

amino acids is proline or hydroxylproline and it lacks tryptophan. Gelatin usually contains 1% sugars derived from mucopolysaccharides and displays two types of ionizable groups: carboxylic acid of aspartic and amine of lysine, arginine and histidine as well as the terminal moieties. Type A gelatin is obtained under acidic hydrolysis and displays an isoelectric point of 7–9. Conversely, type B gelatin is obtained in basic medium and the isoelectric point is 4.5–5. The viscosity of gelatin depends on type, concentration, pH, temperature and time and the gel strength is measured in Bloom, varying from low (<150) to high (>220) Bloom types. The gelation mechanism is still unclear though it is hypothesized that small portions in some gelatin molecules form crystallites that serve as crosslinking points of a 3D network that immobilizes water. Interactions would involve hydrogen bonds, van der Waals forces and peptide linkages. Gelatin has also showed the ability to form films. The presence of the arginine–glycine–aspartic acid (RGD) sequence confers this material cell adhesion properties through integrins together with its good biocompatibility has paved the way to the profuse research of gelatin in cell culture and tissue engineering applications [294].

Similarly, due to its good mucoadhesiveness, it has been explored for the development of drug delivery matrices and use by different administration routes alone or in combination [21,295–298]. Further, chemical modifications have been pursued to improve the performance. For example, Wang et al. synthesized aminated gelatin microspheres to improve the retention in the stomach with respect to the pristine protein [299–301].

4.2. Synthetic polymers

4.2.1. Poly(ethylene glycol) and poly(ethylene oxide) and its copolymers

PEG/poly(ethylene oxide) (PEO) is a highly biocompatible poly(ether) [302] that is cleared by renal filtration [303]. Depending on the synthetic pathway, whether it is polycondensation of ethylene glycol or addition reactions of ethylene oxide, two products with identical chemistry though very different molecular weight and properties can be produced.

The mucoadhesive properties of PEG/PEO are controversial because of the lack of side functional groups (*e.g.*, amine, carboxylic acid) that can specifically interact with components of mucin [304], though the mechanism would rely on the fast interpenetration of PEG chains with the lining mucus layer [305]. The performance also depends on the administration route and the flux of biological fluids. Some groups have compared its performance with more popular mucoadhesive polymers such as CS [306]. In addition, there have been several attempts to improve the adhesiveness of PEG by its modification with PAA [307,308]. Cu et al. modified the surface of poly(lactic-*co*-glycolic acid) (PLGA) acid NPs with PEG to increase the retention time upon vaginal administration with positive results [112]. On the other hand, none of the marketed vaginal mucoadhesive gels contains PEG [309].

Following this rationale, other copolymers that contain PEG and display additional features such as responsiveness to environmental stimuli have been explored. This is the case of poly(ethylene oxide)-*co*-poly(propylene oxide) (PEO–PPO) block copolymers [310]. Aqueous solutions of these biomaterials form gels upon heating and produce only minor irritation following administration by different parenteral routes [311]. These biomaterials are commercially available in two architectures, the linear poloxamers (Pluronic®) and the branched poloxamines (Tetronic®) (Table 4), and a broad spectrum of molecular weight and hydrophilic–lipophilic balances [310]. The former are only thermo-responsive, while the latter are also pH-sensitive.

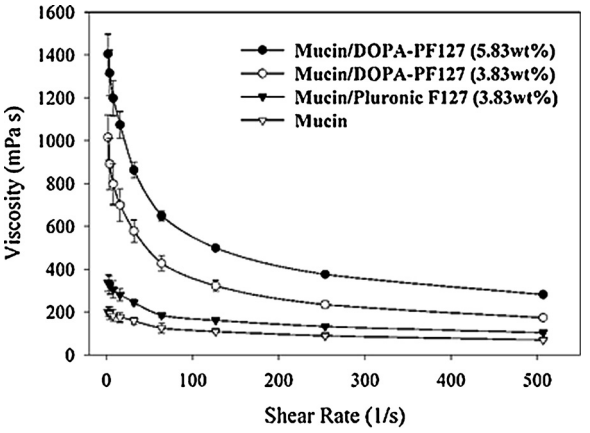

Fig. 5. Effect of pristine and DOPA-modified Pluronic® F127 on the viscosity of mucin from bovine submaxillary glands as a function of shear rate (1/s) at 25 °C. The concentration of bovine submaxillary mucin was constant at 6.55 wt% for all samples. The viscosity of 3.83 wt % solutions of DOPA-PF127 and Pluronic F127 were less than 1 mPa [319], Copyright 2002.
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Poloxamer and poloxamine gels usually display poor physical stability in contact with fluids due to the low microviscosity of the generated networks. Thus, the group of Cohn investigated different chemical modifications that increased the performance of PEO–PPO copolymers as matrices for drug delivery [312–316]. To the abovementioned, work of Bromberg that modified different linear derivatives with PAA blocks [307,317,318], other approaches that include chemical modification and blending with have been introduced to optimize the mucoadhesiveness of these copolymers. These systems were envisioned for different administration routes. Huang et al. used a different approach and modified poloxamers with 3,4-dihydroxyphenyl-L-alanine (DOPA), an amino acid found in mussel adhesive proteins [319]. Assays of interaction of the modified copolymer with bovine submaxillary mucin showed the sharp increase of the viscosity, indicating the ability of the new material to interact with the glycoprotein (Fig. 5) [319].

Blends are a relatively simple strategy to combine the properties of different polymers. Bilensoy developed a vaginal gel of Pluronic® F127 (20%) with low concentrations of mucoadhesive polymers (*e.g.*, poly(acrylate)s and HPMC) for the localized release of the antifungal drug clotrimazole [320]. However, mucoadhesion was not tested. Majithiya et al. used a similar composition for the nasal delivery of sumatriptan [321]. The mucoadhesive force estimated as detachment stress was determined

Table 5
Characterization and transport of unmodified and PEGylated dendron gene vectors in cystic fibrosis sputum.

Gene vector formulation	Hydrodynamic diameter (nm)[a]	ζ-Potential (mV)[b]	$MSD_w/\langle MSD\rangle$[c]
PAMAM/DNA	52 ± 1	34 ± 2	9000
dPEG-PAMAM/DNA	73 ± 3	−0.2 ± 0.8	110
PEI/DNA	33 ± 1	32 ± 1	9700
dPEG-PEI/DNA	44 ± 4	6 ± 1	60

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[a] Measured by dynamic light scattering. Error values represent the standard error of measurement (SEM) of three independent measurements.

[b] Measured in 10 mm NaCl pH 7.1. Error values represent SEM of three independent measurements.

[c] MSD_w is the theoretical mean squared displacement of particles in water calculated from the Stokes–Einstein equation and using the relation MSD = 4Dt, at a time scale of t = 1 s. ⟨MSD⟩ is the ensemble-averaged mean squared displacement of particles in CF sputum measured at a time scale of 1 s. The ratio $MSD_w/\langle MSD\rangle$ indicates by what multiple the average particle transport rate is slowed in CF sputum compared to that in pure water. The larger the ratio, the higher the degree of hindrance to particle motion. PAMAM: poly(amidoamine); PEI: poly(ethylene amine).

using sheep nasal mucosal membrane and increased with growing concentrations of poly(acrylate). More recently, others used similar approaches for the mouth [322].

As mentioned above, PEG has been also used to confer mucus-penetration properties to different types of nanocarriers [98,323]. The low molecular weight and the high surface-modification density are fundamental to minimize the electrostatic and the hydrophobic interactions with mucus, the phenomenon being more remarkable in larger NPs (Fig. 6) [46]. These studies support the improved transport of tetanus toxoid encapsulated within poly(lactic acid) (PLA) NPs coated with PEG across intestinal and nasal mucosae [324]. This approach was useful to prepare non-viral gene vectors that penetrate human mucus barriers (*e.g.*, sputum) (Table 5) [323].

4.2.2. Poly(acrylic acid) and poly(methacrylic acid) derivatives

PAA, also known as carbomer, is a high molecular weight polymer of acrylic acid used as a viscosity modifier in semi-solids and as matrix for classical tablets (Table 4). Due to the good biocompatibility, they have been approved for use in non-parenteral pharmaceutical products [174]. Due to the presence of pendant carboxylic acid units (one per repeating unit), PAA exhibits very high adhesive bond strength in contact with tissues, enhancing the mucosal penetration of drugs; the presence of a unionized carboxyl group is critical in the formation of a strong interaction with mucus. These interactions are thought to be a result of the hydrogen bonds between PAA and the proton-accepting groups in mucin [325]. Due to the presence of numerous carboxyl groups, PAA likely adopts a more favorable macromolecular conformation and an increased accessibility of its hydrogen-bonding groups when compared to other polymers. Additionally, due to its ability to control the release of drugs, PAA and, mainly its derivatives combining several substituted repeating units (*e.g.*, poly(methylacrylate)), have been extensively exploited as polymeric excipients for the development of conventional DDS for non-parenteral administration. However, their specific application for NPs is still limited, being now under consideration for the production of colloidal carriers.

To fine tune the properties of PAA, some research groups have synthesized copolymers of PAA and PEG. PEG has been reported to act as an adhesion promoter between PAA and mucin by linear diffusion of the PEG chains into the acrylic networks and the mucin layer [326]. In this context, PAA–PEG NPs have been reported to improve the transcorneal diffusion of pilocarpine [327] and possess excellent *in vitro* antitumor activity to drug-sensitive as well as drug-resistant cancer cells [328]. In another work, papain/PAA blend NPs were used to study the transport through intestinal porcine mucus compared to unaltered PAA NPs. Results demonstrated a strongly enhanced permeation performance with respect to pure PAA counterparts owing to the local disruption of mucus by papain [329]. Improved transport rates, reduction in mucus viscosity and the delayed release of hydrophilic macromolecular compounds make proteolytic enzyme functionalized NPs very promising to improve the targeted drug delivery of drugs at different mucosal surfaces [329]. Later, it was demonstrated that the majority of the papain functionalized PAA NPs were able to cross the mucus layer and remained in the duodenum and jejunum, where the absorption of the drug primarily occurs [330]. In a similar approach, cysteine/PAA microparticles increased the permeation of vitamin B12 across the intestinal mucosa [331], taking advantage of the thiolated particles compared with unmodified PAA ones. The group of Bromberg has extensively investigated the modification of linear PEO-PPO block copolymers [310], with PAA terminal segments to confer the copolymer mucoadhesive features and assessed these new materials in different DDS, including gels, matrices and polymeric micelles [332–334].

Poly(methacrylates) are hydrophobic synthetic polymers composed of pristine and modified methacrylic acid repeating units with good degree of biocompatibility, though very limited biodegradability (Table 4). In this context, a broad variety of derivatives commercially available as Eudragit® have been developed. These copolymers have been approved by regulatory agencies for use in medical devices and usually non-parenteral pharmaceutical products [335]. For example, the main components of hard and soft contact lenses are poly(methyl methacrylate) (PMMA) and poly(hydroxyethylmethacrylate) (PHEMA), respectively. Thus, these copolymers in general and PMMA in particular have been widely employed as excipients in the development of classical controlled release pharmaceutical dosage forms. More recently, some authors have suggested their biocompatibility for parenteral routes [336–338]. In fact, PMMA is the main component of conventional and medicated bone cements used in hip replacement interventions [339]. In more recent past, they have been explored for the production of NPs, although due to its bio-inertness the use as colloidal carrier has been somehow neglected. PMMA-based NPs can be prepared either by the direct polymerization of the MMA monomer or from pre-formed polymers of different molecular weight by

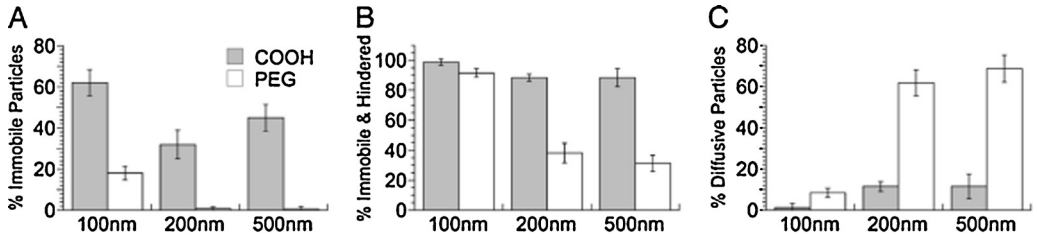

Fig. 6. Transport mode distributions of COOH- and PEG-modified particles in cervical mucus: immobile particles (*A*), immobile and hindered particles (*B*), and diffusive particles (*C*). Data represent mean ± SD of three experiments, with $n > 120$ nanoparticles for each experiment. Immobile particles have a mean squared displacement (MSD) below the microscope detection limit (10 nm) for entire length of video [46], Copyright 2007.
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emulsion/solvent evaporation or nanoprecipitation techniques [340].

Despite the advantageous mucoadhesive properties, PMMA has been reported to show an incomplete drug release, possibly due to its hydrophobic nature. To improve drug release, recent strategies are focusing on increasing polymer hydrophilicity by synthesizing functionalized PMMA with carboxylic functional groups or by formulating PMMA composites with hydrophilic polymers [340]. An alternative approach consists in enhancing drug diffusion within the polymer matrix by including plasticizers in the formulations.

So far, there have been few attempts to encapsulate proteins or DNA in PMMA NPs. The incorporation of bovine para-influenza type 3 virus (BPI-3) proteins was reported using PMMA NPs as vaccine carriers [341]. Higher levels of virus-specific antibody have been reported in comparison to soluble viral proteins alone. Cationic aminoalkylmethacrylate copolymer NPs were evaluated for their use as potential anionic antisense oligonucleotide carriers [342]. A significant portion of adsorbed oligonucleotides were protected from enzymatic degradation. The cellular uptake of oligonucleotides into Vero cells was significantly enhanced. To further adjust the features of the delivery system to the application, NPs of the amphiphilic PMAA-grafted-PEG were prepared by dispersion polymerization and assessed for the oral administration of calcitonin [343]. These NPs exhibited pH-sensitivity release, suitable for gastrointestinal administration [344] and demonstrated to be safe to the intestinal mucosa [345]. Similar NPs were prepared by emulsion polymerization for DNA vaccine applications [346]. The NPs reversibly adsorbed large amounts of DNA, mainly through electrostatic interaction, preserved its functional structure, efficiently delivered it intracellularly, induced significant antigen-specific humoral and cellular responses and greatly increased Th1-type T cell responses, and cytotoxic T cells against HIV-1 Tat and were non-toxic both *in vitro* and *in vivo*. The combination of poly(metahcrylate)s with other polymers is another approach to develop nanocarriers with more tuned properties. For example, PMMA NPs surrounded by a cationic branched poly(ethyleneimine) (PEI) shell were synthesized *via* a graft co-polymerization of methyl methacrylate from branched PEI to encapsulate plasmid DNA [347]. PEI is able to condense DNA into compact particles and protect it from enzymatic degradation. Those NPs internalized and released the plasmid DNA into HeLa cells very efficiently, with less toxic effects than DNA associated with PEI alone [348]. More recently, Seremeta et al. encapsulated the antiretroviral efavirenz within NPs of pure polycationic poly(methacrylate), a derivative that binds to mucin through electrostatic interactions, and blends with poly(epsilon-caprolactone) (see below) [349,350]. Overall these copolymers have become key players in the development of mucoadhesive nano-DDS.

4.2.3. Poly(vinyl pyrrolidone)

Poly(vinyl pyrrolidone) (PVP), also usually referred to as povidone, is a non-ionic synthetic linear polymer comprising groups of 1-vinyl-2-pyrrolidinone (Table 4). The polymer is soluble in various aqueous and organic solvents, chemically inert and essentially non-toxic for non-parenteral exposure [351]. PVP is manufactured by radical polymerization of vinylpyrrolidone (Reppes synthesis) in a range of different molecular weights ($\approx 1–10^3$ kg/mol), being characterized by their *K*-value. PVP is widely used in the pharmaceutical industry as an excipient for a wide range of solid and liquid dosage forms for oral, mucosal and topical administration, although licensing of parenterally-administered medicines containing PVP has been granted by the US FDA. It also finds application in cosmetics and as food additive.

Although traditionally used for its mucoadhesive properties [297], PVP has been described as possessing only mild mucoadhesive properties. Indeed, Ivarsson and Wahlgren have recently reported on the mucoadhesive properties of PVP at intestinal pH (6.6) using different *in vitro* methodologies [352]. These authors found that no interactions were apparent when PVP (molecular weight = 40 kg/mol) and mucin were mixed in solution as assessed by ellipsometry, and tensile strength and rheology methods, thus supporting that this polymer is not mucoadhesive. These results were in contrast to those obtained in another study, where ALG beads coated with PVP (molecular weight = 1.1×10^3 kg/mol) were shown to interact with mucin. The beads also presented a mild mucoadhesive in cell-based adhesion and pig gastric mucosa *ex vivo* wash-off tests. Apart from evident differences in used methodology, which can significantly impact the evaluation of adhesion [353,354], the physical form and molecular weight of PVP may have an impact on its behavior. Overall, the mild adhesive properties of PVP seem to limit its usefulness for

the development of mucoadhesive dosage forms comprising mixtures of different polymers [355–357].

4.2.4. Poly(vinyl amine)

Poly(vinyl amine) or poly(aminoethylene) is a hydrophilic linear cationic synthetic polymer (Table 4) currently produced from poly(*N*-vinylformamide) by alkaline hydrolysis [358]. It has been used experimentally as a component of DDS, particularly as a condensing agent of genetic material for intracellular delivery [359,360]. The application of the mucoadhesive properties of poly(vinyl amine) has been limited to the production of NPs presenting the polymer at their surface [361] or drug–polymer conjugates [362]. For example, Sakuma et al. observed that the intestinal transit time of poly(vinyl amine)-coated polystyrene NPs containing a ^{125}I-derivative radiotracer in rats was significantly increased when compared to a control (radiotracer in a solution of PEG), thus supporting that NPs were mucoadhesive [361]. The mucoadhesive nature of this polymer is most probably related with its cationic nature and the ability of amine groups to interact with negatively charged mucin.

4.2.5. Boronate-containing polymers

Boronate-containing copolymers (BCCs) are synthesized by the copolymerization of 3-aminophenylboronic acid (APBA) precursors (Table 4) obtained by the modification of the amine group with different monomers such as *N,N′*-dimethylacrylamide [363]. These materials have been shown to display LLR binding activity [364–366] and, thus, they have attracted attention as potentially synthetic mucoadhesive biomaterials. Water-soluble BCCs showed specific polysaccharide-binding capacity [366] and formed insoluble complexes with mucin due to boronate–sugar interactions [367,368]. This unique feature has been used to occlude a mucosal lumen with a poly(vinyl alcohol) gel [369] and to propose new techniques for cell separation that rely on the interaction of the cellular membrane and boronate moieties [370,371] and targeting strategies to specific tumoral cells [372–375].

4.3. Semi-synthetic polymers

Aiming to fine tune the properties of natural polymers, a broad range of chemically-modified derivatives have been developed.

4.3.1. Cellulose derivatives

The polysaccharide cellulose is the most abundant biopolymer in nature, comprising a linear chain of β(1 → 4) linked *D*-glucose units (Table 4). Cellulose is one of the most widely used excipients in the production of medicines, either in the powder or microcrystalline forms, and is obtained from fibrous plants by purification and mechanical size reduction of α-cellulose [174]. Alongside its insolubility in water and most organic solvents, cellulose presents various other functional and technological limitations that can be systematically abbreviated by the semi-synthesis of different ether and ester derivatives [376]. Most commonly used cellulose ethers include methylcellulose (MC), ethylcellulose (EC), hydroxyethyl cellulose (HEC), HPC, HPMC, and CMC salts – calcium (CaCMC) or NaCMC (Table 4) [174]; as for esters, cellulose acetate and cellulose acetate phthalate are the most frequently used in pharmaceutics. In general, the most commonly used cellulose ethers and esters for topical and mucosal drug delivery are considered as non-toxic and non-irritating materials, including some GRAS listed ones [174].

Different cellulose derivatives have been described as mucoadhesive polymers [377–379]. A considerable number of studies compared the mucoadhesive potential of cellulose derivatives, among them and with other polymers, by using various conditions and methodologies [380]. However, most studies fail to fully characterize the properties (*e.g.*, degree of substitution and molecular weight) of the cellulose derivative used. Thus, a straightforward and systematic analysis of their relative mucoadhesive performance is difficult but a brief overview for a few cellulose ether derivatives is presented in the following paragraphs.

Hydroxyethyl cellulose. HEC presents considerable mucoadhesive potential at pH 6.8 but usually lower than that of HPC and NaCMC [285]. Efforts to improve its mucoadhesion include the thiolation of native HEC. In a study by Sarti et al., thiolated HEC was achieved by substitution of hydroxyl groups of the unmodified polymer with thiourea *via* nucleophilic substitution of a bromo-HEC intermediate [381]. The time of adhesion of this new polymer in the form of tablets to pig intestinal mucosa attached to the rotating cylinder of a dissolution apparatus at pH 6.8 and 37 °C was assessed. The adhesion time of thiolated HEC was 4-fold longer than that of HEC, presumably due to the establishment of disulfide bonding with mucin. In another recent report by the same group, a cationic HEC-cysteamine conjugate was prepared through partial ring opening of glucose units of HEC using sodium periodate, followed by reductive amination/thiolation with cysteamine [382]. This derivative (3%, *w/v*) was shown to interact better with mucin than HEC under intestinal conditions (pH 6.8), as determined by rheological methods. The presence of thiol groups and positively-charged amine groups are presumably responsible for this effect, even if a reduction in solubility also occurred. A follow-up study also showed that HEC-cysteamine NPs obtained by cross-linking with tripolyphosphate presented higher mucoadhesion potential than those obtained with oxidized HEC-cysteamine (*i.e.*, conjugate presenting lower availability of free thiol groups), thus confirming the role of disulfide bridging in the establishment of adhesive interactions with mucin [383].

Hydroxypropyl cellulose. HPC presents high mucoadhesive potential. Adhesive behavior in the solid state to pig intestinal mucosa was shown to be comparable to that of several poly(acrylate)s (Carbopol® 971 and 974, and polycarbophil) at pH 6.8 when 300 kg/mol HPC was used [285]. When compared to other cellulose derivatives, it presented similar to higher and higher mucoadhesive performance than NaCMC and HEC, respectively [285]. The adhesive forces established between HEC gels and mucin disks at pH 6.8 did not produce relevant differences when various concentrations of the polymer were used (5–12%, *w/v*) but increasing time of contact between both systems showed

to be essential in increasing adhesion [384]. Further modification of HEC may be valuable in increasing mucoadhesive performance, namely by introducing cationic groups in order to enhance electrostatic interaction with mucin. For example, diethylaminoethyl-HEC was shown valuable in increasing the mucoadhesive properties of HEC as assessed by turbidimetric titration, zeta potential measurements and a mucin particle method [385].

Hydroxypropyl methylcellulose. HPMC has been reported as possessing good mucoadhesive properties in the solid state. For instance, this cellulose derivative in the form of compressed disks performed better than polycarbophil, Carbopol® 934 and NaCMC in a tensile test using rat intestinal mucosa at pH 6 [386]. Also, its mucoadhesive behavior was not affected by pH in the range of 4–8 but decreased to roughly half when the pH of the medium decreased to 2.2. However, another study revealed that the mucoadhesiveness of HPMC is also dependent of the type of mucosa considered: for example, HPMC (86 kg/mol) disks were shown to be more mucoadhesive to duodenal bovine mucosa than to sublingual or esophageal mucosae; also, when compared to Carbopol® 974P disks, HPMC was more or less mucoadhesive in the case of duodenal or sublingual mucosae, respectively [26]. These results further highlight the difficulty of a direct comparison between different literature reports and advise a case-by-case analysis when considering the choice of a mucoadhesive material and/or DDS. Moreover, they stress that *ex vivo* assays are of limited value to predict the performance of mucoadhesive systems *in vivo*. When used in the liquid state (0.33–0.67%, *w/w*), the capacity of HPMC to interact with mucus seems to be noticeably reduced, as assessed by rheological methods, but still in range with that of ALG [387]. Indeed, a study conducted by Sigurdsson et al. using highly diluted polymer solutions ($\leq$0.01%, *w/v*) showed that no interactions between HPMC (86 kg/mol) and mucin were observed in the pH range of 4.0–8.2 by using a resonant mirror biosensor [283]. Further studies seem to backup that HPMC is unable to bond to mucin, being its mucoadhesive behavior probably related with chain entanglement and physical interlock with mucus [284,388].

Sodium carboxymethyl cellulose. NaCMC has been reported to present only mild mucoadhesive properties. For example, when compared to other polymers such as HPMC, polycarbophil and Carbopol® 934, NaCMC in the solid state showed 2–5-times shorter adhesion time to *ex vivo* rat intestinal mucosa, as determined using a tensile methodology at pH 6 [386]; contrasting with these findings, a report by Bogataj et al. showed that polymeric films of NaCMC presented similar force of detachment from pig vesical mucosa to that of polycarbophil films but lower than CS ones [389]. Also, NaCMC gels (3%, *w/v* in water) presented higher ability to interact with mucin than similar HPC and HPMC gels, as assessed by a rheological synergism method [390]. Despite these apparent conflicting results, it is consensual that different properties (*e.g.*, molecular weight) of NaCMC can influence its mucoadhesive performance. Molecular weight should be optimized to promote adequate polymer flexibility and interpenetration with mucin [391]. Indeed, physical interlocking with mucin chains is the most probable mechanism that explains the mucoadhesive behavior of NaCMC [284]. Moreover, pH seems to influence the adhesive behavior of NaCMC. For example, the interaction of the polymer (140 kg/mol) with mucin was shown higher at low pH (4); as pH increases, adhesive interactions seem to decrease down to become nearly absent at pH 5.5 [283]. These observations made using a resonant mirror biosensor were correlated by the authors with the pK_a of NaCMC ($\approx$3.5): above this point the polymer becomes increasingly ionized and formed negative charges lead to the establishment of repulsive forces with the also negatively-charged mucin chains. Another aspect influencing mucoadhesion is the polymer hydration status. Lower time of pre-hydration was shown to result in higher mucoadhesion, presumably due to the higher degree of polymer–mucin interpenetration promoted by faster diffusion of water into the dry polymer matrix upon mucin/mucosa contact [391]. Also, the progressive reduction of polymer concentration at the gel formed in the adhesive interface with increased pre-hydration time may be involved as shown by Jones et al. by using NaCMC gels of different concentrations and testing their adhesive force to mucin disks [384]. Indeed, the NaCMC interaction with mucus seems to be minor when the fully hydrated polymer is used [387]. The mucoadhesive performance of NaCMC in the solid state was also shown highly dependent on its physical form; Grabovac et al. found that the polymer after precipitation resulted in higher mucoadhesion values as compared to freeze-dried NaCMC at pH 6.8 [285]. In the same study, polymer pre-treatment with different pH values (3 or 7) did not seem to affect notoriously mucoadhesive behavior. Also, thiolation of this cellulose derivative (NaCMC-cysteine) was shown generally effective in increasing mucoadhesion.

Methylcellulose and ethylcellulose. MC presents only very mild ability to interact with mucin when diluted in water (0.67%, *w/w*) [387]. EC has been described as presenting poor to low mucoadhesive potential. In one study evaluating the mucoadhesive properties of polymeric NPs by different methods, EC exhibited mixed behavior [392]. Even so, considerable adhesion could be inferred from experiments testing the immobilization of NPs at mucin-coated glass plates or *ex vivo* pig gastric mucosa. Also, the mucoadhesive performance of NPs comprising mixtures of EC and MC was slightly improved as assessed by the same methodology.

5. Main pharmaceutical applications of mucoadhesive polymers in nano-DDS

This section discusses each non-parenteral administration route and the main therapeutic benefits of making a system mucoadhesive. Since nanomedicine has emerged as a groundbreaking field to prevent, diagnose and treat disease, only works at the crossroads of mucosal administration and nanotechnology will be addressed.

5.1. Oral administration

Oral administration is the most patient-compliant, especially in the therapy of chronic maladies [393] because it is painless, non-invasive, does not require specialized

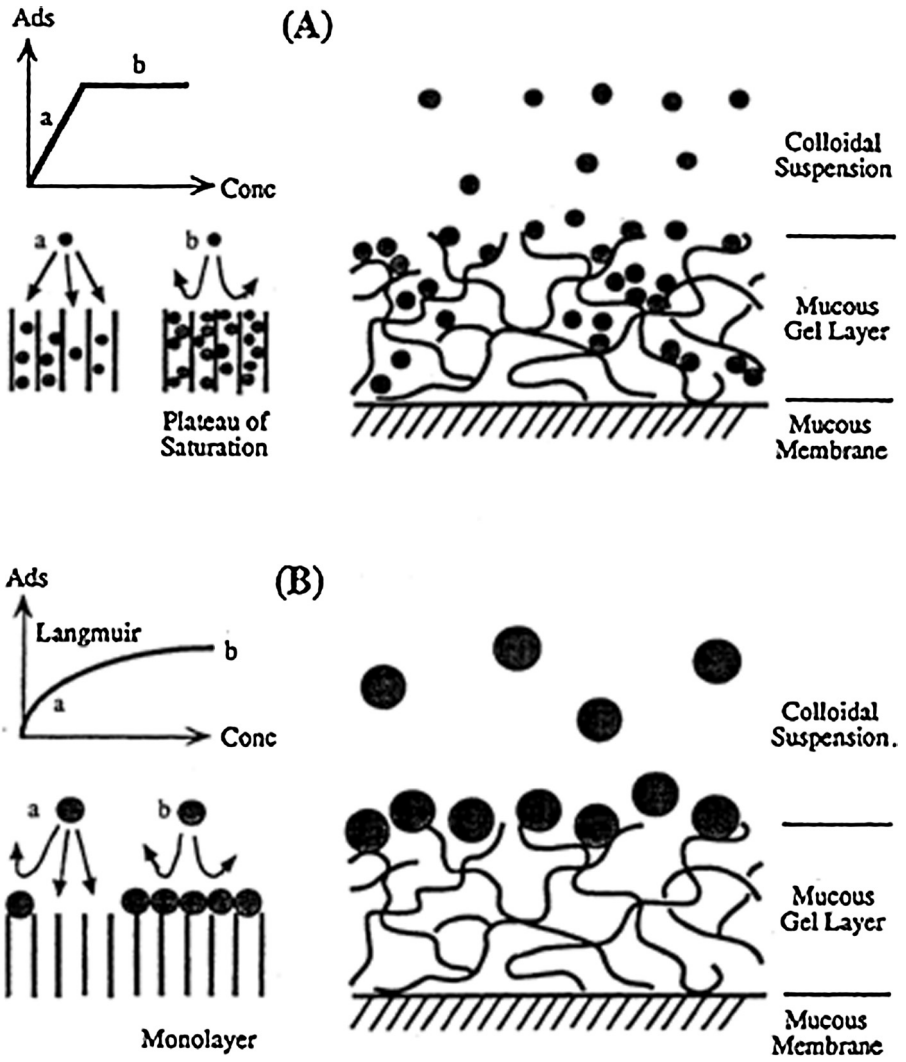

Fig. 7. Adsorption isotherms and corresponding adsorption models. A: particles <1 μm. B: particles > 1 μm [94], Copyright 2001. Reproduced with kind permission from Elsevier Ltd.

personnel and enables self-administration [394]. These advantages are of special interest for the treatment of pediatric patients [395]. In this context, the field of mucosal administration has been led by the development of DDS for oral administration. However, to achieve this goal, nanocarriers need to be appropriately engineered to display mucoadhesive features.

Nanosuspensions produced by the dispersion of pure drug NPs of hydrophobic drugs by bottom-up or top-down technologies are probably the simplest nanotechnology applied to pharmaceutical sciences and it is entailed mainly to increase dissolution rates and, by doing so, to improve bioavailability [8,396,397]. Pure drug NPs have been surface-modified with polymers such as chitosan to prolong their retention in the GIT tract and to increase the therapeutic efficacy [398].

Following this basic concept, further works went one step forward and focused on the development of different polymeric and lipidic nanocarriers modified with CS and PAA, two of the most well-investigated mucoadhesive polymers [399]. Progresses made have been of relevance not only for the administration of physicochemically stable drugs but especially for those most sensitive to the GIT environment, such as peptides and proteins [399–401]. The effect of the different structural features on the interaction of the nanocarrier with the layer of mucus have been investigated, as represented in Fig. 7, for drug carriers of variable size [94]. In general, the smaller the size, the more intimate this interaction and the more prolonged the residence time and the drug oral bioavailability. However, predictions are complex due to the involvement of additional parameters. Thirawong et al. produced pectin/liposome nanocomplexes to improve the oral bioavailability of calcitonin [402]. Pectin modification increased the size of the liposomes and prolonged the residence time of the DDS in the gut over at least 6 h, reducing calcium plasma concentrations to a greater extent than the free protein solution (Fig. 8) [402]. Another

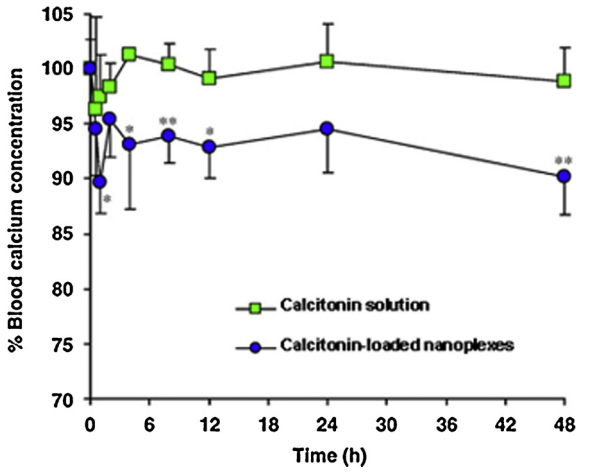

Fig. 8. Plasma calcium concentration after intragastric administration of calcitonin solution and pectin/liposome nanocomplexes in the dose of 500 IU/kg rat. The means and standard deviations of three measurements are shown. $^{*}p < 0.05$; $^{**}p < 0.01$, significantly difference from calcitonin solution [402], Copyright 2008.
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property that is relevant to adhesion is surface charge. Usually, positively-charged NPs are more adhesive to mucin than negatively-charged ones (Fig. 9) [399]. The intragastric administration of calcitonin-loaded liposomes coated or not with CS or PAA to rats and the monitoring of the calcium concentration in plasma confirmed the benefit of the positively-charged surface [403]. Thus, positively-charged liposomes reduced the calcium concentration to a greater extent than the negatively-charged ones, the effect being more prolonged for CS-coated liposomes. However, this behavior also depends on the integrity of the intestinal wall and some works indicated that the injury of the mucosal layer in specific medical conditions such as inflammatory bowel disease (IBD) might expose proteins of the subjacent layers of the intestine that favor the adhesion of electronegative particles over the electropositive ones [404]. Calcitonin-loaded CS-modified PLGA NPs showed a similar improvement with respect to the uncoated counterpart [405].

Another approach to achieve mucoadhesion relies on the surface modification of NPs made of non-adhesive biomaterials with non-ionic polymers such as PEG derivatives that penetrate the mucus layer or CS derivatives that interact directly with mucin, as reported for poly(cyanoacrylate) NPs [406,407]. Jin et al. nano-encapsulated thymopentin, a model pentapeptide that induces early T cell differentiation and immune regulation and that has been used in the therapy of the acquired immunodeficiency syndrome (AIDS), cutaneous T-cell lymphoma/cancer immunodeficiency and rheumatoid arthritis, within poly(butyl-cyanoacrylate) (PBCA) NPs coated with CS and CS-glutathione [407]. All the NPs by the oral route showed a beneficial effect on inmunosuppressed rats, suggesting that the absorption of the peptide was improved by just nanoencapsulation. In addition, the benefit was remarkable for systems modified with mucoadhesive polymers, especially CS-glutathione. Sajeesh and Sharma sophisticated the delivery system and encapsulated an insulin/cyclodextrin complex within NPs made of PMAA, CS and PEO–PPO copolymers [408]. The mucoadhesiveness was tested in excised rat intestinal mucosa. Results showed the retention of more than 84% of the NPs even after several washings with buffer solution. Despite the positive results, it is worth stressing that reproducibility is crucial at the time of bench-to-bedside translation and that the combination of various polymers obtained from different sources added to complex

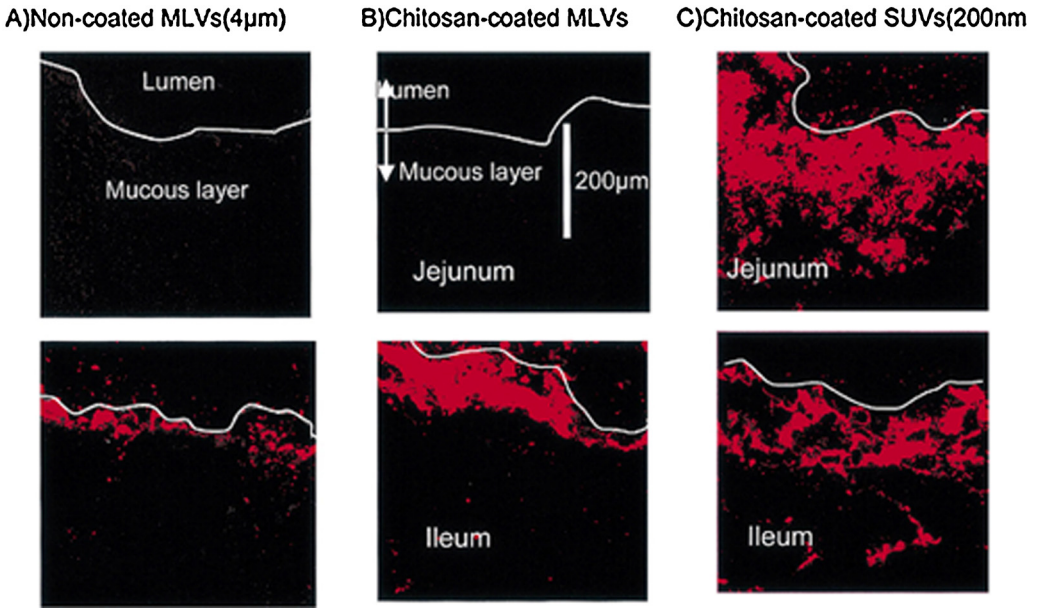

Fig. 9. Confocal laser scanning micrographs of uncoated and chitosan-coated (A,B) multilamellar vesicle (MLV) and (C) small unilamellar vesicle (SUV) liposomes 2 h after intragastric administration. The particles are 4.6 μm (A) and 339.2 nm (B), respectively [399], Copyright 2001.
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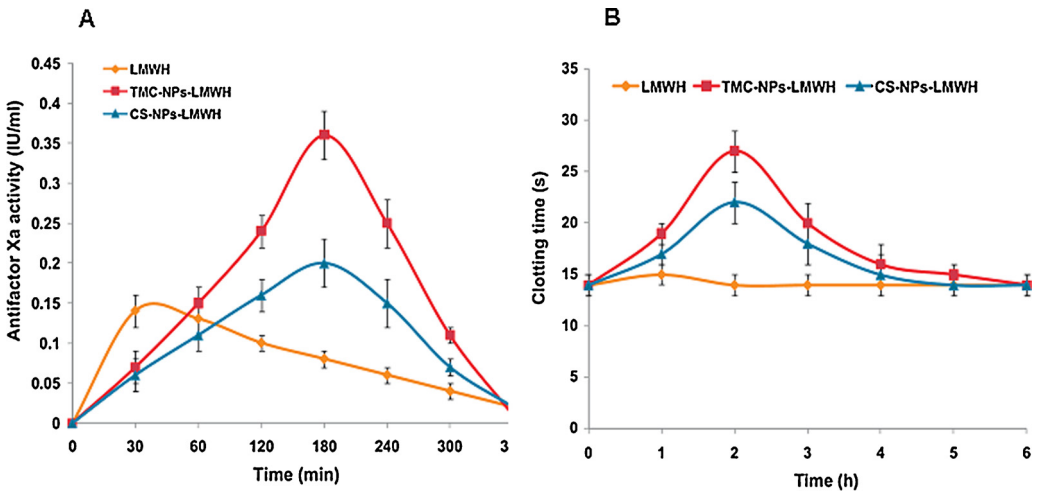

Fig. 10. (A) Antifactor Xa activity *versus* time profiles of different LMWH loaded CS-NPs and TMC-NPs formulations after oral administration in equivalent dose of 50 mg/kg. Data represents mean ± SD ($n = 6$). (B) Clotting time profile of LMWH loaded CS-NPs and TMC-NPs formulations after oral administration in equivalent dose of 50 mg/kg. Data represents mean ± SD ($n = 6$–8) [417], Copyright 2012.
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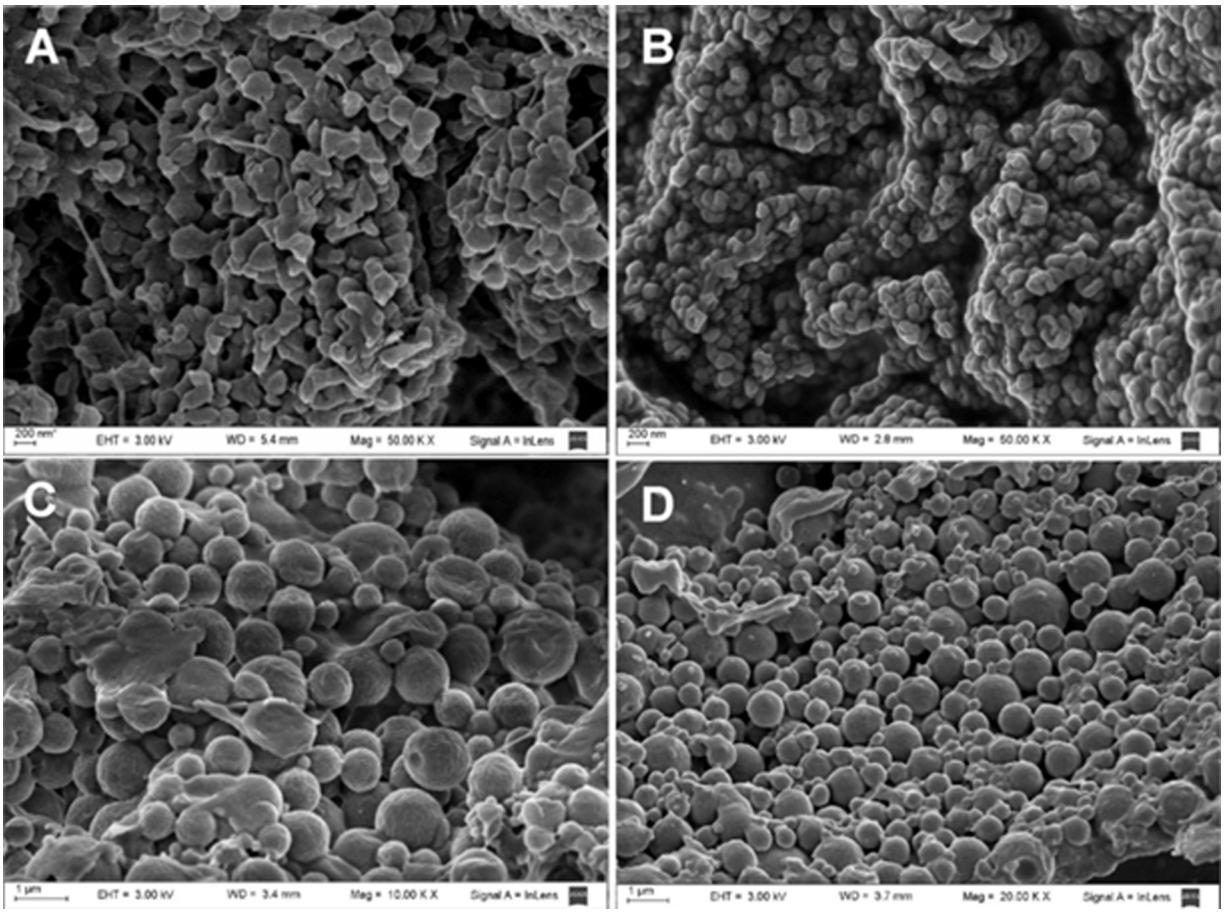

Fig. 11. SEM micrographs of efavirenz-loaded (A–C) and efavirenz-free particles (D). (A) Pure low molecular weight PCL (PCL_L), (B) high molecular weight PCL/Eudragit® RS 100 (1:1) (PCL_H-RS), (C) low molecular weight PCL/Eudragit® RS 100 (1:1) (PCL_L-RS) and (D) EFV-free low molecular weight PCL/Eudragit® RS 100 (1:1). Ratios are expressed in weight by weight. Scale bar: (A) and (B) = 200 nm; (B) and (C) = 1 μm [350], Copyright 2013.
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production methods might challenge this task, if not preclude it. Trapani et al. also explored a platform of hydrophilic cyclodextrins (*e.g.*, carboxymethyl-β-cyclodextrin and sulfobutyl ether-β-cyclodextrin) and CS NPs for the delivery of both hydrophilic and hydrophobic drug cargos [409]. Systems were also made more complex by the incorporation of additional components. For example, Reis et al. developed a novel DDS for the oral administration of insulin that was based on an ALG/dextran sulfate core complexed with a CS/PEG/albumin shell [410]. Insulin-loaded NPs were administered to diabetic rats in doses between 25 and 100 IU/kg by the oral route. Findings indicated the sustained decrease of glycemia over 24 h with a maximum effect 14 h after the administration. In addition, after 4 days, a dose of 50 IU/kg improved the diabetic status with a decrease of the water intake, urine excretion and proteinuria. More recent works employed other CS derivatives (*e.g.*, lauryl succinyl CS) [411] and polymer combinations with similar results [412]. By far, insulin has attracted most of the attention in this niche [413–416].

The delivery of other biologically active molecules that are usually administered by injection (*e.g.*, low molecular weight heparin, LMWH) within CS NPs has been investigated [417]. Even though the performance of these systems was worse than the i.v. injection, it was significantly better than that of free heparin; *e.g.*, it augmented and delayed the antifactor Xa effect and the plasma clotting time (Fig. 10) [417]. This effect was more remarkable for TMC NPs that were retained by the intestinal mucin for more prolonged times than the unmodified counterpart owing to the pH-independent nature of the quaternary ammonium cationic moieties. Other studies assessed the encapsulation of cyclosporine [418] and efavirenz [350] within polymeric NPs containing commercially available poly(acrylate)s of the Eudragit® series.

The group of Sosnik developed the first aqueous pediatric formulation of the antiretroviral efavirenz, a first-line drug used in the infection by the human immunodeficiency virus (HIV) [419–423] employing simple and mixed polymeric micelles – a kind of self-assembly nanocarrier – made of pristine and chemically modified linear and branched PEO–PPOs [310]. Preclinical studies in rats showed a significant increase of the oral bioavailability with respect to a suspension and an oily solution. However, since polymeric micelles undergo gradual disassembly upon dilution in the GIT fluids, they do not sustain the release over time. To prolong the release over time, the drug was encapsulated within PCL, PCL/Eudragit® RS 100 and Eudragit RS® 100 NPs (Fig. 11) [350]. In addition, incorporation of Eudragit® increased the agglutination in presence of mucin *in vitro*, leading to a fast growth of the hydrodynamic diameter (D_h), as measured by dynamic light scattering (DLS) (Table 6) [349].

Conversely, pure PCL NPs did not show changes. Moreover, the release was sustained over at least 1 week (Fig. 12) [350]. More importantly, preliminary pharmacokinetics studies showed that Eudragit® RS 100 reduced the burst effect, resulting in more uniform plasma concentrations that were detectable for longer times (Fig. 13) [349]. In addition, PK parameters showed that pure

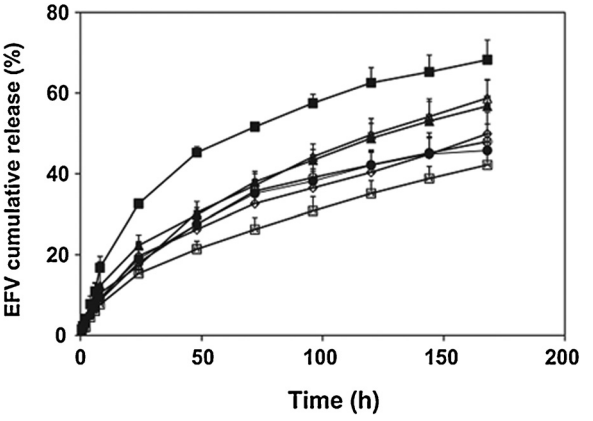

Fig. 12. EFV cumulative release from different nanoparticles prepared by nanoprecipitation and simple emulsion/solvent evaporation over one week. PCL_L (■), PCL_H-RS (1:1) (△), PCL_L-RS (1:1) (▲), PCL_L-RS (1:3) (◊), PCL_L-RS (emulsion with ethylacetate) (1:1) (○), PCL_L-RS (emulsion with dichloromethane) (1:1) (●) and Eudragit® RS 100 (RS) (□). Data are reported as mean ± SD ($n = 3$) [350], Copyright 2013.
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Eudragit® RS 100 increased the oral bioavailability of the drug with respect to pure PCL and PCL/Eudragit® RS 100 blend NPs due to increased mucoadhesion (Table 7). Conversely, blend NPs showed a slight non-statistically significant decrease with respect to PCL counterparts. The oral bioavailability was governed by two phenomena: (i) the residence time of the NPs in the gut (due to mucoadhesion or not) and (ii) the release rate. While the incorporation of Eudragit® increased mucoadhesion (Table 6), it also decreased the release rate of the encapsulated drug. These results would indicate that, in the case of blend NPs, the mucoadhesion *in vivo* was not strong enough to compensate the decrease in the release rate, resulting in a bioavailability drop. Conversely, in pure RS NPs, mucoadhesion probably prolonged the residence time and enabled the more complete release of the encapsulated drug.

The encapsulation of alendronate, a drug used for the prevention and/or treatment of post-menopausal osteoporosis that shows an extremely poor oral bioavailability of 0.6% within liposomes coated with chitosan was assessed by Han et al. [424]. Coating significantly increased the oral bioavailability of the drug with respect to the free molecule and uncoated liposomes (Table 8).

LLRs are sugar-binding transmembrane proteins that are highly expressed in cells of the immune system [233] and that have been used to actively target drugs to monocytes/macrophages by the administration of sugar-modified nanocarriers [234,235,237,238]. Due to their high affinity for glycosylated structures of the mucus, a few works addressed the surface modification of different nanocarriers with soluble lectins (*e.g.*, wheat germ agglutinin) to confer mucoadhesiveness [425,426]. For example, Sharma et al. followed this approach to improve the oral pharmacokinetics of several co-encapsulated antituberculosis drugs [425]. It is worth mentioning that the cost of this modification is relatively high and the scalability unlikely, what could preclude its implementation at a larger (*e.g.*, industrial) scale.

Table 6
Hydrodynamic diameter, polydispersity index (PDI) and ζ-potential of efavirenz-loaded NPs incubated in mucin solution for 2 h. Composition ratios are expressed in weight by weight [349].

Nanoparticle	$T = 0$ h			$T = 2$ h			S_F/S_I
	D_h (nm) (±SD) Peak	PDI (±SD)	ζ-pot (mV) (±SD)	D_h (nm) (±SD) Peak	PDI (±SD)	ζ-pot (mV) (±SD)	
PCL_L	235.0 (1.3)	0.093 (0.013)	−11.2 (0.8)	242.4 (1.7)	0.113 (0.013)	−17.6 (0.0)	1.03
PCL_H-RS (1:1)	93.02 (0.74)	0.115 (0.009)	+31.1 (1.2)	1162 (110.1)	0.347 (0.125)	− 2.2 (0.8)	12.49
PCL_L-RS (1:1)	153.2 (2.4)	0.117 (0.015)	+38.7 (1.8)	967.6 (115.8)	0.281 (0.054)	−5.8 (0.9)	6.31
PCL_L-RS (1:3)	140.6 (2.4)	0.137 (0.010)	+33.4 (2.2)	882.9 (156.1)	0.498 (0.138)	0.0 (0.8)	6.28
RS	139.0 (6.1)	0.254 (0.008)	+45.8 (2.8)	948.7 (97.9)	0.410 (0.035)	+2.8 (0.7)	6.82

PCL_L: PCL of low molecular weight, 14 kg/mol.
PCL_H: PCL of high molecular weight, 80 kg/mol.
RS: Eudragit RS® 100. S_F/S_I: Size ratio after (S_F) and before (S_I) incubation with mucin.

Table 7
Pharmacokinetic (PK) parameters of efavirenz after oral administration of 40 mg/kg to rats ($n = 4$) [349]. CV%: Coefficient of variation

PK parameter	PCL_L		PCL_L–RS		RS	
	Mean	CV%	Mean	CV%	Mean	CV%
C_{max} (μg/mL)	1887	37.49	2330	29.17	1587	8.39
t_{max} (h)	2.50	40.00	3.25	29.46	3.75	40.00
AUC_{0-24} (μg/mL/h)	13.93	25.64	14.53	31.40	19.42	32.12
AUC_{0-24} (μg/mL/h)	35.99	36.09	21.11	30.46	60.13*	27.32
k_e (h^{-1})	0.04	30.33	0.05	42.29	0.02	16.80
F (%)	100.00	N.D.	58.65	N.D.	167.07	N.D.

* Statistically significant increase of the parameter between pure Eudragit® RS 100 and PCL NPs ($p < 0.05$).

Mucosal vaccination by the oral route has become an area that attracted a great deal of attention during the last years [25,427–429]. Due to its unique combination of properties, CS remains one of the key players in this research topic [430], often combined with ALG. For example, Borges et al. evaluated the immune response after oral vaccination with hepatitis B antigen (hepatitis B surface antigen, HBsAg subtype ADW2) encapsulated within ALG-coated CS NPs [431]. ALG coating was aimed to stabilize the particles and prevent immediate desorption of the antigen in the GIT medium, and favor the uptake by the M cells of the Peyer's patches in the gut [432]. The effect of the antigen alone was compared to the performance of a combination with a synthetic oligodeoxynucleotide containing the immunostimulatory CpG motif as adjuvant, and in association (or not) with the NPs. Groups that were administered HBsAg and HBsAg/adjuvant presented improved immune response with greater CD69 expression in CD4+ and CD8+ T-lymphocytes and lower in B lymphocytes [431]. A similar interest is found in CS for oral gene delivery [433].

5.2. Inhalatory administration

The airways provide a very large absorption bed that can be advantageous not only in the treatment of pulmonary diseases (*e.g.*, asthma) and overcoming local infectious diseases [434] due to the restriction of systemic exposure and adverse effects, but also for the systemic delivery of drugs in the so-called transpulmonary route. At the same time, the potential of this alternative route has promoted the development of novel aerosol technologies that maximize the administered dose and the deposition level [435]. Inhalation pharmaceutical products must fulfill a number of features that include aerosol particles with mean aerodynamic diameter between the 0.5 and 5 μm to favor deposition in the deep lung, aerosol particles with low size distribution and high reproducibility, dissolution or adhesion to the lining mucosa and appropriate drug release and permeability [20]. In this context, different nano-DDS have been conceived for inhalatory administration [436]. Surprisingly, the research at the interface of nano-DDS for inhalation and mucoadhesion is elusive [437] and the reports countable. In two different works, the group of Lehr showed the beneficial effect of lecithin to increase the adhesion of liposomes to alveolar macrophages (Fig. 14) [438,439]. Others coated different nanocarriers with PAA, CS [399] and HPC [440]. Since tuberculosis (TB) is primarily an infection localized in the lungs, Khuller extensively assessed the potential of the

Table 8
Excreted drug amount in urine after an oral administration of alendronate in different formulations (mean ± SD, $n = 3$–4).

	Total drug amount excreted into urine (μg)	% of dose excreted into urine
Chitosan-coated liposome	38.6 ± 4.5*	3.4 ± 0.5*
Uncoated liposome	21.6 ± 4.1*	2.1 ± 0.4*
Non-liposome	14.1 ± 4.3	1.3 ± 0.4

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* $p < 0.05$, compared to the control group (non-liposome).

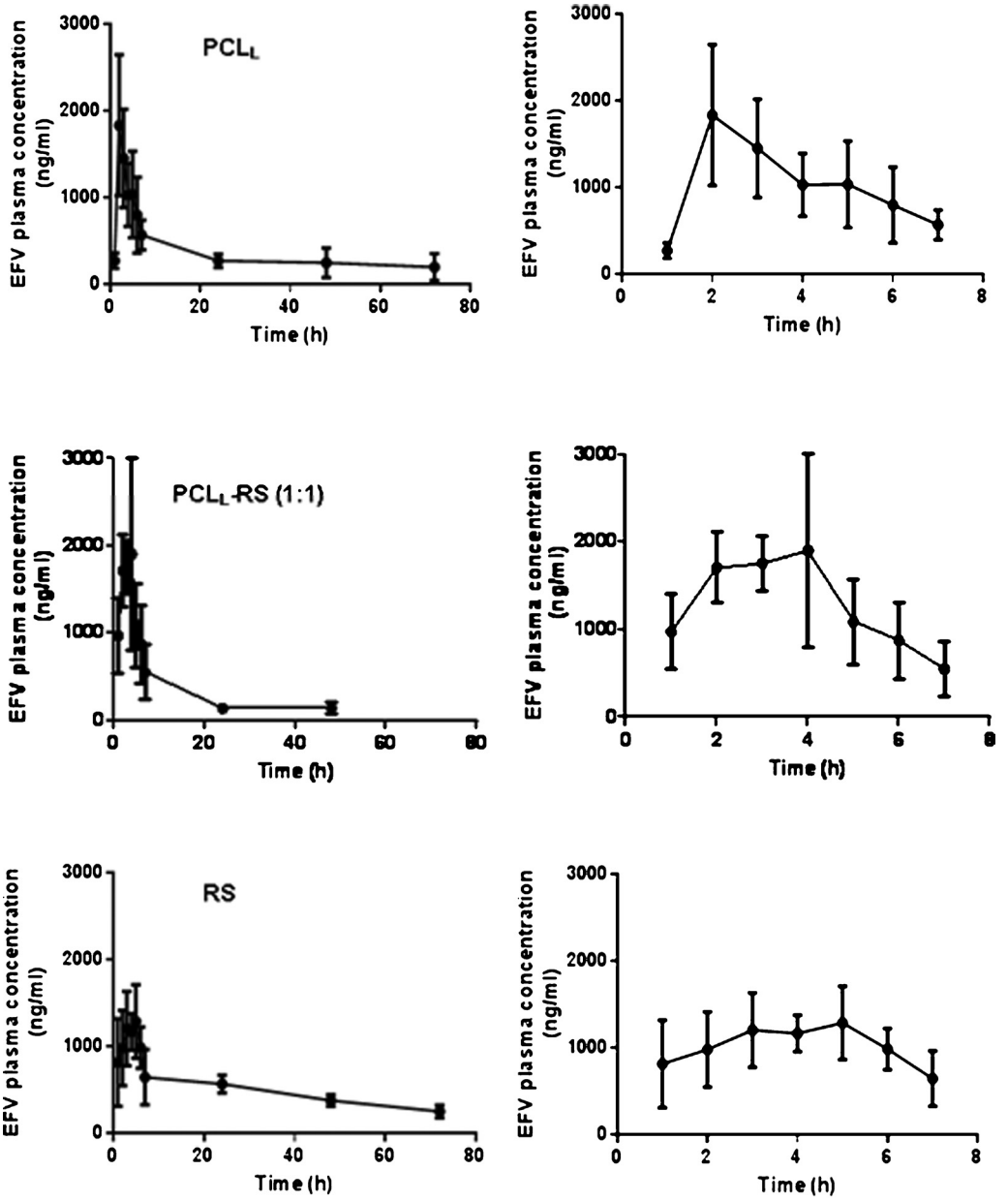

Fig. 13. Efavirenz plasma concentration after the administration of efavirenz-loaded nanoparticles by the oral route (20 mg/kg), over 48 h ($n = 4$) [349].

inhalation route to treat the pulmonary form of TB by employing different inhalable dry powders loaded with first-line anti-TB drugs [425,441,442]. This approach was also advantageous to actively target alveolar macrophages, the intracellular TB reservoir of the mycobacterium [443]. Following this trend, our group has successfully nanoencapsulated the anti-TB drug rifampicin within "flower-like" polymeric micelles [444] and used this platform to develop a liquid rifampicin/isoniazid combination that showed improved oral bioavailability of rifampicin [445]. The coating of these polymeric micelles with CS and hydrolyzed GalM conferred them recognition and mucoadhesive properties [238]. These novel nano-DDS showed significantly greater drug uptake by macrophages *in vitro* [238] and good aerosolization ability [446], thus opening new therapeutic opportunities to treat this global health threat.

5.3. Ocular administration

The eye is a complex and sensitive organ, consisting of three main layers, the outer coat or the sclera and cornea, a middle layer or uveal coat and the inner coat or retina [189]. The sclera is made of fibrous tissues shaped as segments of two spheres, the sclera and cornea. From the drug absorption point of view, cornea and conjunctiva represent the two major mucosal barriers that drugs must cross to reach the possible local of actions. The cornea is a clear, transparent, avascular tissue to which nutrients

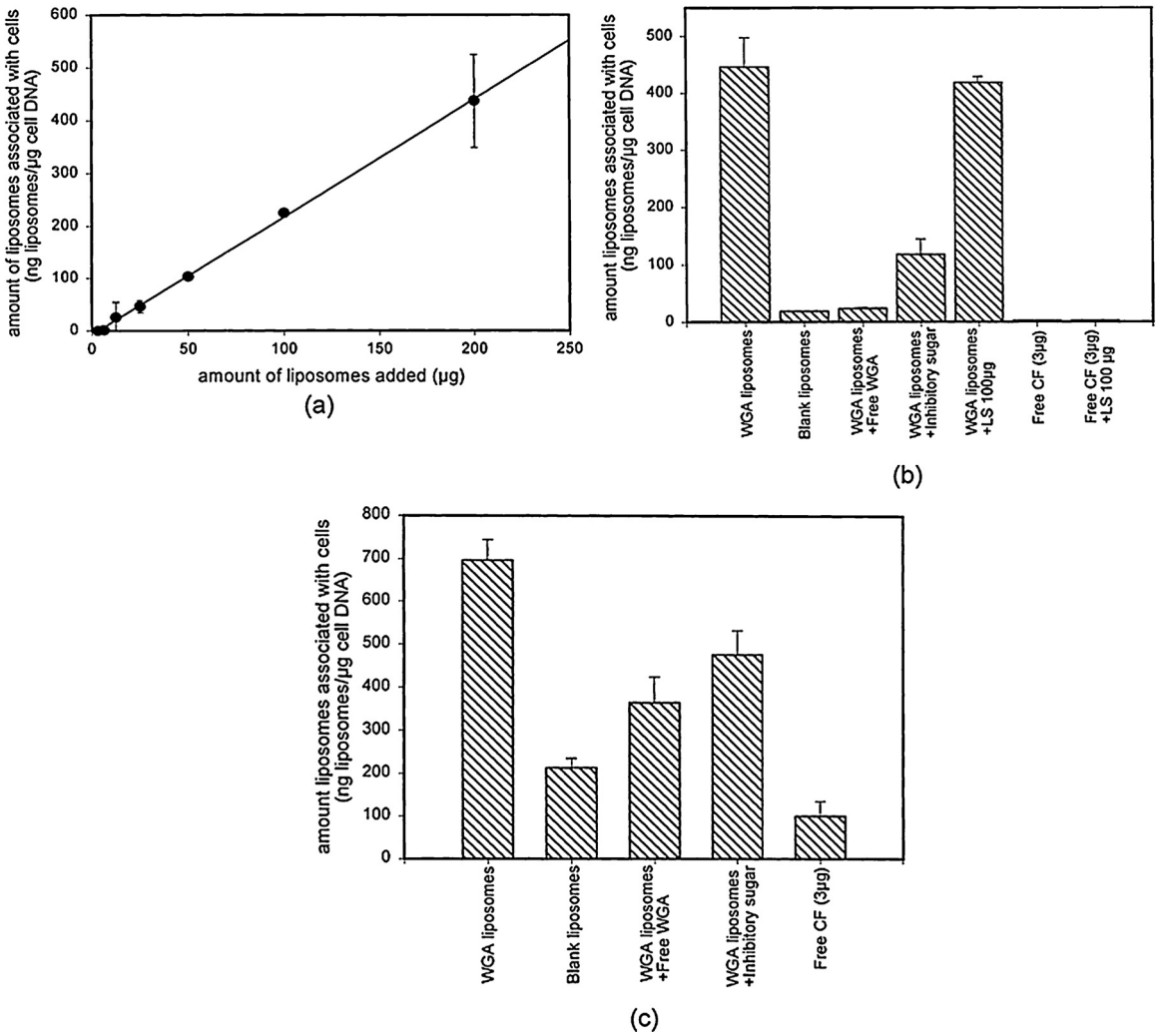

Fig. 14. Interaction of liposomes modified with the lectin wheat germ agglutinin (WGA) with alveolar epithelial cells. (a) Relationship between the amount of liposomes added to A549 cells and the amount associated after 90 min incubation. 24-well plate. (b) Cell association of 200 mg WGA liposomes with A549 cells. WGA liposomes, WGA-functionalized liposomes. Blank liposomes, dipalmitoylphosphatidylcholine (DPPC):cholesterol liposomes. WGA liposomes + free WGA, WGA liposomes and 20-fold free WGA. Inhibitory sugar, 20 ml of 20.0 mM diacetylchitobiose. LS, Alveoafact (lung surfactant). (c) Cell association of 100 mg WGA liposomes with primary human alveolar cells. Results represent the average and standard deviation of at least three determinations from two different passage numbers for A549 cells and two independent preparations for primary human alveolar cells [439], Copyright 2001.
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and oxygen are supplied by the lachrymal fluid and aqueous humor. The corneal epithelium consists of 5–6 layers of columnar cells squeezed forward by the new cells. Replacement of the epithelial cells occurs by mitotic division of the basal layer every 4–8 days [189]. The conjunctiva is a thin transparent membrane, which lines the inner surface of the eyelids and is reflected onto the globe. At the corneal margin, it is structurally continuous with the corneal epithelium. Conjunctival epithelium is composed by 5–7 cell layers connected by tight junctions, which render the conjunctiva relatively impermeable. The membrane is vascular and moistened by the tear film [189]. Despite the apparent easy accessibility, the eye is well protected from foreign materials by several efficient mechanisms forming a physical–biological barrier, such as blinking, induced lacrimation, tear turnover, naso-lacrimal drainage, which cause rapid removal of drugs from the eye surface and from the back cornea [447]. Additionally, the blood–retinal-barrier (BRB) and the extra ocular epithelia represent an obstacle in the drug delivery to the choroid, retina, and vitreous. Only a fraction of the drug administered orally or by subcutaneous or intramuscular routes reaches the retina, requiring large doses to be therapeutically effective [448]. Moreover, approximately 95% of the administered drug is removed by the tears and does not reach the site of action or, conversely, is absorbed into the systemic circulation leading to adverse effects. In this context, DDS for topical ocular administration are an interesting and promising approach to treat eye diseases, especially because they are a non-invasive way of

releasing drugs in a controlled fashion directly to a specific compartment of the eye and because they prolong the residence time of the drug in the site of action and reduce the amount of drug that is absorbed by alternative routes [189,449]. Among the possible strategies for ocular drug delivery, which include biocompatible viscous solutions and film-forming gels [450], liposomes [451], solid lipid NPs (SLNs) [451], microspheres [452] and medicated-contact lenses [453], the use of biodegradable nanocarriers has been considered a very promising system [454], though scarcely capitalized until now. In ophthalmic applications, it is convenient that particulate systems have an appropriate size, preferably within the nano-range, in order to avoid irritation, foreign body sensation, and discomfort to the patients [455]. Other factors depending on the success of NP systems for ocular drug delivery lays on optimizing lipophilic–hydrophilic properties of the polymer–drug system, optimizing rates of biodegradation, and safety. However, the highly sensitive corneal/conjunctival tissues require great caution in the selection of the carriers toward eye penetration to maximize drug transport.

Different biomaterials have been used to prepare NPs, such as poly(acrylates), PLA, PLGA, dextran, ALG, collagen, hyaluronic acid and CS [449] and their ocular application evaluated.

CS has been investigated as a superior mucoadhesive cationic polymer due to its ability to develop molecular attraction forces by electrostatic interactions with the negative charges of mucin, as mentioned before. CS NPs may encapsulate a wide range of drugs for ocular purposes, maintaining their biological activity as antibacterial [104] or anti-inflammatory agents [456]. CS NPs are also able to interact and remain associated to the ocular mucosa for extended periods of time [457] and after inoculation in the rabbit ocular surface, no signs of inflammation or alteration were observed [456]. Simultaneously, it was confirmed that CS NPs are taken up by conjunctival and corneal epithelia *in vivo*. CS NPs crosslinked with sulfobutylether–cyclodextrin were developed to encapsulate econazole, presenting sustained drug release and better *in vivo* antifungal effect in rabbits compared to the free drug for 8 h [458]. CS NPs have also been exploited to develop gene delivery systems to the eye, taking advantage of the synergistic mucoadhesive and transfection enhancing properties of the polymer. As example, to determine whether CS NPs would be suitable for intraocular use, pDNA carrying the ubiquitously expressed CBA-eGFP expression cassette was compacted administered to adult wild-type albino mice; CBA-eGFP is a vector in which green fluorescent protein (GFP) reporter gene expression is driven by chicken b-actin (CBA) heterologous promoter. At day 14 post-injection, substantial GFP expression was observed exclusively in the retinal pigment epithelium in eyes treated with the loaded NPs but not in those treated with pDNA or the vehicle [459]. Moreover, no signs of gross retinal toxicity were observed, and there was no difference in electro-retinogram function between NPs, pDNA, or vehicle-treated eyes. In a similar approach, formulations of CS-DNA NPs were administered to rat corneas as model animal resulting in luciferase gene expression 5 times greater than following administration of PEI-DNA NPs [460]. Even though these formulations were not assessed in topical administration, they open new research avenues toward less invasive ophthalmic therapies.

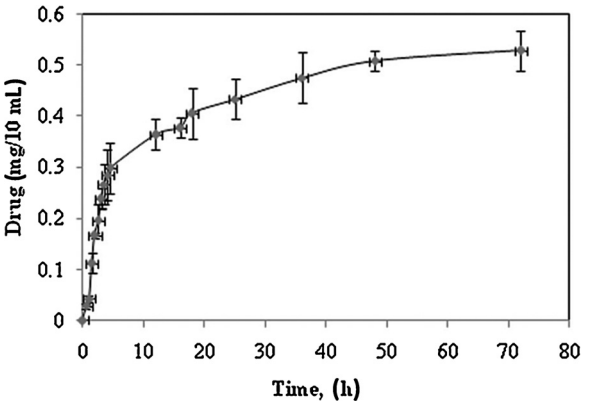

Fig. 15. *Ex vivo* release kinetics of pilocarpine hydrochloride for PAA-PEG particles [327], Copyright 2014.
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PLGA and PLGA–PEG NPs were used to encapsulate melatonin, a neuro-hormone secreted by the pineal gland able to modulate intraocular pressure [461]. Topical application of melatonin formulations caused ocular hypotension in rabbit eyes, thus emerging as an alternative approach to treat glaucoma. The maximum effect (5 mmHg), which was obtained with the PLGA–PEG formulation, occurred at 2 h and persisted up to 8 h, with a significant difference compared to melatonin aqueous solution and PLGA NPs, showing that mucoadhesion generally prolongs the contact time of a formulation with the eye surface [461]. PLGA NPs were also used to deliver cyclosporine A to the eye, for the treatment of inflammation of the rabbit eye surface as model animal [462]. The cytotoxic effect of NPs was found to be time and concentration dependent and also showed significantly higher degree of cellular uptake, tear film concentration of the drug and double bioavailability values in comparison with the drug emulsion [462].

Another polymer used to develop NPs for drug delivery was PAA. Cross-linked particles based on PAA and PEG with nanometer size and spherical shape loading pilocarpine demonstrated enhanced drug release and permeability into the corneal mucus stratum because of NP assembly and mucoadhesion (Fig. 15) [327].

Recently, polymeric micelles of PEO–PPO have been evaluated for the encapsulation of the anti-glaucoma agent ethoxzolamide [463]. However, this delivery system is not mucoadhesive, which represents a limitation for this administration route. However, the use of higher concentrations of these thermo-responsive copolymers would enable both the nanoencapsulation of the drug and the formation of a gel upon contact with the ocular mucosa.

It is now well-established that polymeric mucoadhesive NPs are able to deliver any drug at the right time in a safe and reproducible manner to a specific anterior and posterior segment of eye at required level. In this scenario, the exploration of more sophisticated mucoadhesive nano-DDS in the coming years is ensured.

5.4. Vaginal administration

The vagina has been long used for drug delivery and constitutes an interesting route (if not preferential in some cases) for the management of local gynecological conditions, such as infection and cancer, or spermicidal contraception [84]. Moreover, due to the good absorption profile of some compounds through its mucosa, vaginal administration may be performed to obtain systemic drug levels that have been shown useful for hormonal contraception or replacement therapy, inducement or prevention of labor, and pregnancy termination. The investigation in the field of vaginal drug delivery has seen a huge boost over roughly the last decade, mostly because of the development of microbicides [464,465]. These last can be defined as products intended to be administered in the vagina (and/or rectum) around the time of sexual intercourse in order to prevent the sexual transmission of HIV and/or other pathogens (*e.g.*, HSV-2, herpes simplex virus that produces most genital herpes). Even if established drug delivery strategies have been preferred for microbicide drug delivery namely comprising the use of vaginal gels, films or rings, nanotechnology-based systems with intrinsic antiviral activity or used as drug carriers have been also extensively investigated, often with promising results [466–471].

The vagina is a tubular organ connecting the cervix to the vestibule and presenting a more or less noticeable slope when women are standing up [472]. This creates a natural tendency for products placed in the vagina to leak to the exterior, particularly during ambulation [473,474]. The use of mucoadhesive drug dosage forms/delivery systems may help abbreviate this issue, and thus potentially optimize therapeutics due to prolonged *in loco* drug residence and increased patient compliance [475–478]. In the particular case of drug nanocarriers, similar advantages seem to be applicable. For instance, Cu et al. showed that mucoadhesive avidin-modified PLGA NPs (150–170 nm) were successful in increasing the system retention upon vaginal administration to mice, as compared to similar unmodified NPs [112]. Although the primary objective of avidin-modification was not the enhancement of mucoadhesion, the presence of this protein at the surface of NPs increased adhesive interactions with mucin as assessed *in vitro*. NPs leaking from the vagina were collected using absorbent paper placed in the floor of cages where mice were freely moving; results showed that the amount of particles leaking after 30 min was reduced almost 5-fold in the case of mucoadhesive NPs, while negligible amounts of NPs were recovered from the vagina at 2 h after administration in all cases (Fig. 16). These results were also correlated with a significant increase in the amount of particles recovered by vaginal washing for mucoadhesive NPs over non-modified ones but not over densely PEG-modified ones [112]. Indeed, the general trend in the field of vaginal drug delivery has been the development of mucus-penetrating nanocarriers [100]. Major advantages of using such systems include: (i) the ability to better distribute throughout the vaginal tract as promoted by facilitated mucus diffusion, (ii) increased transport across the mucus barrier toward the underlying mucosal tissue, where drugs can be directly delivered to target cells (*e.g.*, immune cells that can be infected by HIV, cancer cells), and (iii) negligible disturbance of the mucin mesh composing vaginal fluids, which can play an important protective role against various pathogens, namely HIV (Fig. 17) [111–113,116,479–482]. Thus, research on vaginal mucoadhesive polymer-based drug nanocarriers has been scarce, being the most significant examples described below.

Meng et al. proposed CS NPs (200–900 nm) as an adequate mucoadhesive delivery system for tenofovir, a non-nucleotide reverse transcriptase inhibitor, to be used in the development of a vaginal product for preventing HIV-1 sexual transmission [483]. The mucoadhesive nature of such nanocarriers was advocated as potentially beneficial in increasing the relatively low window of protection predicted for the drug once delivered in the vagina. The mucoadhesive potential of NPs was characterized *ex vivo* by simply immersing a strip of pig vaginal mucosa into a dispersion of fluorescently labeled NPs in a simulated vaginal fluid (pH 4.2, as described by Owen and Katz [86]). The amount of NPs retained in the mucosa was indirectly measured by assessing fluorescence in the remaining dispersion of NPs. Results were shown variable according to particle size: higher retention (≈12–14%) was obtained for NPs in the range of 200–300 nm, while larger ones (≈900 nm) presented a near 2-fold decrease. As detailed previously in this manuscript, the reduced ability of larger NPs to penetrate below the top layer of mucus may help explaining these differences [100]. A recent report by the same group also showed that the use of tenofovir-loaded thiolated CS NPs (CS-thioglycolic acid-conjugate, 200–250 nm particles) resulted in increased mucosal retention by around 4- to 5-fold as compared to similar sized CS NPs [484]. Mucoadhesion was time- and concentration-dependent, with maximum retention being observed for CS-thioglycolic acid NPs after 2 h incubation at 1 mg/mL particle concentration (roughly 65% retention), as assessed by the immersion method described above. These observations are presumably related with the required time-lag for disulfide bridging to occur between the thiolated polymer and mucin chains and the saturation of mucin sites where bonding may be established. Overall, these results for CS and thiolated CS NPs seem promising regarding improved vaginal drug retention, even if the consequences of using these permeability enhancing polymers [485] to the barrier effect of the epithelium against HIV transmission are not clear.

Additionally, different researchers reported on mucoadhesive polymer-coated NPs for the development of vaginal microbicides although no formal demonstration of mucoadhesion or mucin interaction was shown. For instance, Lara et al. described PVP-coated silver NPs (30–50) [486], while oligomannose-coated gold NPs (1–2 nm) were proposed by Martinez-Ávila and coworkers [487,488]. Also, Alukda et al. prepared tenofovir-loaded SLNs and modified their surface with poly(*L*-lysine)/heparin by electrostatic layer-by-layer assembly method [468]. In the previous examples, the surface presence of mucoadhesive polymers seems to assure that proposed nanosystems present the ability to interact with mucin and, presumably, increase their vaginal retention

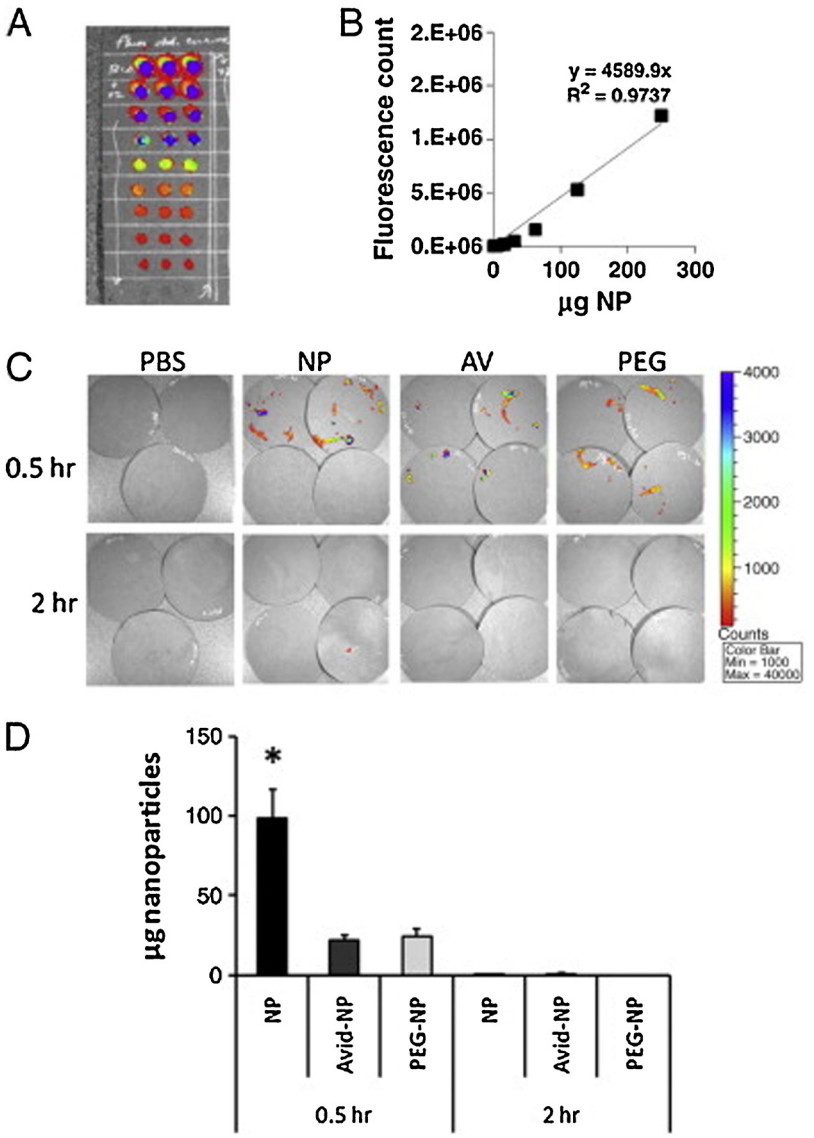

Fig. 16. Measurement of particle leakage from mice after vaginal administration. (A) Fluorescence particles deposited on black absorbent paper were visualized using an IVIS 200 system (Xenogen). (B) A standard curve made from known amounts of particles was used to convert measured fluorescence signals to μg NPs. For each animal, the paper lining was changed once after 30 min, and this second lining was removed at 2 h post initial time of delivery. (C) Images were taken in sets of 3–4 papers (NP, unmodified NPs; AV, avidine-modified NPs; PEG, PEG-modified NPs). Between 20 and 100 μg nanoparticles were found on absorbent papers within the first 30 min, no particles were found on the paper collected after 2 h. (D) The unmodified NPs (indicated as NP) formulation displayed the highest degree of leakage, approximately 5-times greater than avidine-modified NPs (Avid-NP) and mucus-penetrating PEG-modified NPs (PEG-NP); (*) denotes significant difference between sample and remaining groups within similar time point ($p < 0.0005$) [112], Copyright 2011.
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over non-modified counterparts. However, further investigations are needed to realize the potential of these strategies.

5.5. Intranasal administration

The nose is a complex organ entailed to perform a variety of functions that range from olfaction to humidification, warming and filtering of the inhaled air before it reaches the trachea and the lungs [489]. The nasal mucosa comprises two layers, the luminal epithelium containing goblet cells that produce the mucus that covers the epithelium and the underlying lamina propria that is rich in blood and lymphatic vessels, nerves, glands and cells of the immune system. Due to the high surface area offered by the nasal mucosa, the high irrigation and the presence of lymphocytes and mast cells, it has been capitalized for local and systemic drug delivery employing different products and devices [490]. However, the small dimensions and the great sensitivity to xenobiotics impose limitations to the kind of drug and DDS that can be implemented; usually, drugs administered by the nasal route must be very potent to

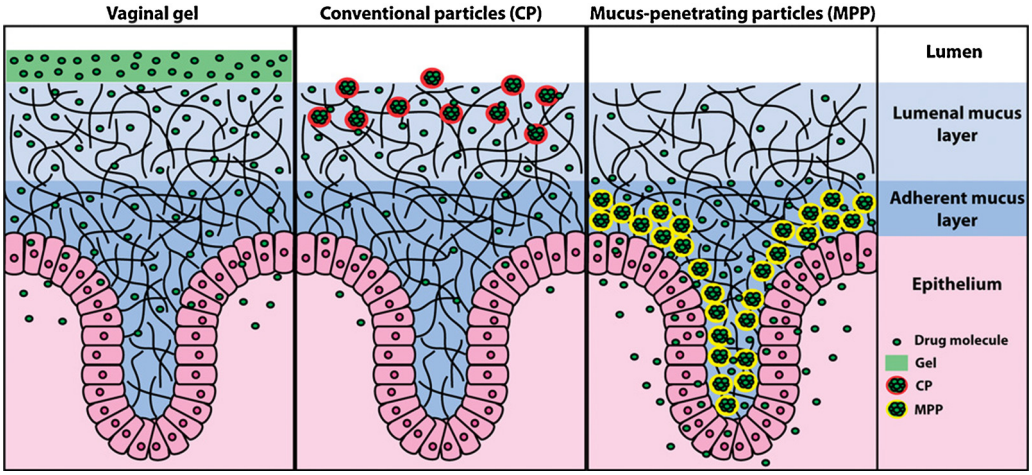

Fig. 17. Graphical depiction of vaginal drug delivery from gel, unmodified or adhesive NP (conventional particles, CP), and mucus-penetrating NP (densely PEG-modified particles, MPP) formulations. Mucus-penetrating NP can diffuse into the deepest mucus layers, namely the more slowly cleared mucus at vaginal *rugae*, thus allowing optimal location for efficient tissue uptake of drug molecule payload [113], Copyright 2012. Reproduced with permission from AAAS.

attain therapeutic concentrations in very small administered volumes. The design of nano-DDS could expand the applicability of this route to other drugs [491]. However, reports on mucoadhesive nano-DDS are almost unavailable. One of the few works was published by Jain et al. that developed mucoadhesive multivesicular liposomes (26–34 μm) coated with CS and Carbopol® for the transmucosal (systemic) delivery of insulin [492]. The carriers contained high protein payloads between 58 and 62%. Furthermore, administration of the mucoadhesive liposomes to streptozocin-induced diabetic rats reduced plasma glucose levels in 35% for 2 days, a better performance than the uncoated ones that reduced them to a similar extent though for only 12 h. It is worth noting that this DDS was also administered by the ocular route with even more promising results; the hypoglycemic effect was observed for 72 h.

The transport of drugs from the systemic circulation into the CNS is constrained by the presence of the blood–brain barrier (BBB) and the blood–cerebrospinal fluid barrier (BCSFB). For decades, these barriers prevented the use of therapeutic agents for the treatment of Alzheimer's disease, stroke, brain tumor, head injury, spinal cord injury, depression, anxiety and other CNS disorders. The greatest challenge faced by developers of new therapeutics for the treatment of diseases of the CNS is overcoming these barriers and the achievement of therapeutic concentrations in the cerebral parenchyma [493]. Direct injection of therapeutic agents into the brain by stereotaxis is possible though this practice entails serious drawbacks associated with the invasiveness of the procedure and the emergence of immunological side effects that limit its application in clinical practice. Attempts to transiently increase the permeability of the BBB (*e.g.*, with mannitol) were also assessed. However, opening the barrier allows the entry of toxins, undesirable molecules and eventually pathogens to the CNS, resulting in potentially significant damage. The capitalization of anatomical pathways represents an appealing approach to localize the drug release and minimize systemic exposure.

The exposure of the intranasal mucosa to environmental NPs and contaminants and their effect on the CNS revealed the presence of a direct nose-to-brain transport pathway that bypasses the BBB and the BCSFB [494,495]. It is interesting to note that the olfactory region is contiguous to the cerebrospinal fluid (CSF) tracts around the olfactory lobe [493]. Drug transport to the brain would be possible through delivery into the olfactory CSF, providing that the molecule is transported across the nasal epithelium and subsequently transported across the arachnoid membrane that separates the sub-mucosal space of the nose and the olfactory CSF [496]. For example, Wang et al. reported on the significant increase of the bioavailability of methotrexate in the CSF with respect to plasma after intranasal (i.n.) administration [497]. Different mechanisms have been proposed for this direct passage though the transcellular one appears as the most relevant, while the paracellular one has been less investigated [494]. In the case of drug-loaded nanocarriers, they would be internalized by the neuronal terminals of the olfactory nerve system that emerge in the brain and end at the olfactory neuronal epithelium. Thus, the i.n. administration of nano-DDS enhances the bioavailability of the cargo in the CNS. Initially, the upper limit for efficient transport across the i.n. mucosa was reported to be 100 nm, though more recently NPs as large as 300 nm were also shown to reach the CNS [498]. Sosnik and coworkers exploited efavirenz-loaded polymeric micelles developed for oral administration [419–423] to target the CNS, one of the most challenging HIV reservoirs, employing the i.n. route. The relative exposure index in CNS was increased up to 3 times with respect to plasma (Fig. 18) [499]. In contrast, the i.v. administration resulted in CNS concentrations significantly smaller than in plasma.

On one hand, the i.n. route presents remarkable advantages such as (i) minimal invasiveness, (ii) painlessness, (iii) self-administration and (iv) high patient compliance [500]. On the other hand, only small volumes can be administered

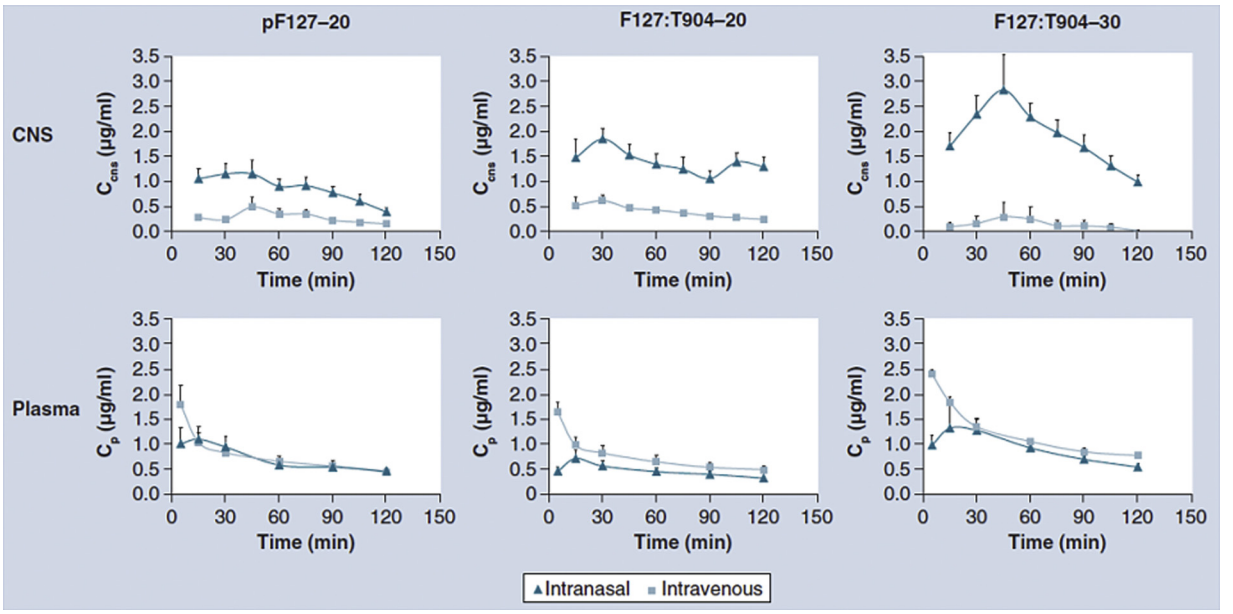

Fig. 18. Plasma and brain efavirenz concentrations after the administration of different PEO–PPO polymeric micelles (20 μL/nostril) by the intravenous and the i.n. routes. Results expressed as mean ± S.E. ($n = 6$) [499], Copyright 2013.
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per nostril at each administration time and only highly concentrated systems can enable the attainment of therapeutic doses. This disadvantage has most likely precluded the bench-to-bedside translation of intranasal products [501]. At the same time, more advanced DDS comprising mucoadhesive nanocarriers as well as repetitive administration regimens could be implemented to overcome this limitation. Kumar et al. reported on the targeting of risperidone to the brain using a mucoadhesive nanoemulsion and compared it to a non mucoadhesive one [502]. Formulations were successfully prepared by the spontaneous emulsification method (titration method) using Capmul® MCM as the oily phase [503]. Mucoadhesiveness was attained by the incorporation of CS. The brain/blood uptake ratio of risperidone was 0.617, 0.754, 0.948, and 0.054 for a solution (i.n.), a nanoemulsion (i.n.), a mucoadhesive nanoemulsion (i.n.) and an i.v. nanoemulsion, respectively, at 0.5 h [502]. These results indicated the direct nose-to-brain pathway. In addition, mucoadhesive systems were more efficient than the non-mucoadhesive ones. Bahadur and Pathak developed nanoemulsions of the antipsychotic drug the ziprasidone hydrochloride [504]. To confer mucoadhesiveness, systems were modified with CS. Khan et al. encapsulated thymoquinone within CS NPs prepared by the ionic gelation method with tripolyphosphate [505]. Based on maximum concentration, time-to-maximum concentration, area-under-curve over 24 h, and elimination rate constant, i.n. thymoquinone-loaded NPs proved more effective in brain targeting compared to i.v. and i.n. thymoquinone solution. Perez et al. developed ^{32}P-siRNA dendriplexes for transfection in the CNS [506]. Even though the nanocarriers were not mucoadhesive, they were incorporated into a mucoadhesive gel of Pluronic® F127 and chitosan or Carbopol® to sustain the release. These works exemplify the great potential of this alternative administration route and probably constitute the beginning of a new era in the therapy of diseases of the CNS.

5.6. Buccal/sublingual administration

Buccal administration comprises the placing of the delivery system in the cheek pouch and the absorption of the drug through the lining mucosa of the oral cavity [507–509]. Sublingual formulations have been developed to be put under the tongue where the drug is released and undergoes fast absorption into the systemic circulation [507,509,510]. The main advantages are rich irrigation and blood flow, thinner mucosa with respect to other body sites (the sublingual being even thinner than the buccal) and increased permeability, limited enzymatic activity and ability to develop unidirectional release systems that minimize oral absorption. Thus, high drug concentrations can be achieved very fast and there is not gastric or hepatic metabolism. On the other hand, the drug can be partially swallowed, which results in a decrease of the bioavailability and a delayed concentration peak.

Due to these advantageous properties a number of researchers have investigated the buccal mucosa for the local and systemic administration of drugs with special interest in proteins and peptides that undergo fast degradation in the GIT employing mucoadhesive NPs [511]. Venugopalan et al. developed mucoadhesive polymeric NPs for the buccal administration of insulin [512]. Studies in diabetic rats showed a significant hypoglycemic response after 7 h, without any detectable fluctuation in blood glucose profile and risk of hypoglycemia. McCarron et al. used drug-free poly(propylcyanoacrylate) NPs to diminish the adhesion of blastospores of *C. albicans*

to human buccal epithelial cells *in vitro* as a strategy to prevent oral candidiasis [513]. However, NPs adhere to the pathogen and not to the oral mucosa. Sandri et al. assessed the penetration enhancement properties of CS and TMC solution and NPs employing excised porcine oral mucosa [514]. The mechanism would involve a repackaging of the epithelial cells up to the basal membrane and a partial disarrangement of desmosomes. In addition, NPs were more efficient than solutions in increasing the permeation of fluorescein isothiocyanate dextran (molecular weight = 4400 g/mol) probably by cell uptake [515]. This *ex vivo* model has been proposed by other groups that used carboxyl polystyrene, amine-modified polystyrene and neutral polystyrene NPs as reference and Franz cells to evaluate the transport route [516,517]. More recently, an advanced *in vitro* model that considered the mucus layer has been proposed [518]. In this case, an external mucus film was deposited on an oral cell line (TR 146) monolayer cultured on Transwells®. Findings showed that porcine mucin is the most similar to the human natural one. In addition, a comparison between this new model and the *ex vivo* porcine buccal excise one indicated that it is reliable. Mazzarino et al. nanoencapsulated the highly hydrophobic curcumin within PCL NPs and coated them with CS, a modification that increased the interaction with mucin *in vitro* [519]. However, the permeation using a model of oral mucosa was not tested, making the study inconclusive. Only a few research groups have explored mucoadhesive colloids for sublingual administration with focus on immunotherapy and vaccination [520]. The oral mucosa is an important site to induce immunological tolerance to protein antigens due to the presence of subsets of tolerogenic dendritic cells in mucosal and submucosal tissues and a small group of Langerhans cells and mast cells [521,522]. Thus, it is well accepted that the oral mucosa is a logical site for immunotherapy and the World Health Organization has pointed out the relevance of this administration route for vaccination [523]. There are a few animal studies with formulations that aim to prolong and facilitate allergen contact with the oral mucosa and uptake by dendritic cells to increase the efficacy of the immunotherapy [521], CS emerging as the most extensively investigated polymer due to its ability to transiently break intercellular junctions.

6. Future perspectives

The capitalization of mucosal tissues has emerged as a promising and solid strategy to improve the bioavailability of drugs, to reduce systemic exposure and to subsequently increase the therapeutic index by means of the design of mucoadhesive nano-DDS. On the other hand, the variability of mucosae and their properties challenge the design of versatile platforms. The broad spectrum of natural, synthetic and semi-synthetic polymers commercially available and (in many cases) approved by regulatory agencies for use in pharmaceuticals enable the adjustment of the properties to the intrinsic features of a specific mucosa and, at the same time, increase the possiblity of technology transfer. However, regardless of the variety of mucoadhesive nanotechnology platforms that have been investigated in academia and the richness of the intellectual property derived from these works, mucoadhesive nano-DDS have not reached the market yet. This situation reveals the difficulties faced to conduct clinical trials, even for more advanced products of already-approved drugs. In this scenario, the coming years will be crucial to consolidate the field and to place the first pharmaceutical products with such features that will support the valuable contribution of these unique nanosystems to treat disease.

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